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ELISHA MITCHELL Scientific Society

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BY RESOLUTION OF

THE ELISHA MITCHELL SCIENTIFIC SOCIETY

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THE NORTH CAROLINA ACADEMY OF SCIENCE

THE TWO ISSUES OF VOLUME 73, 1957

ARE DEDICATED

TO THE MEMORY OF

ELISHA MITCHELL

BORN 1793

DIED 1857

MEMORIAL SERVICE
on the Hundredth Anniversary
of the Death of Dr. Elisha Mitchell

PROGRAM

Invocation ..... *Dr. Frank E. Ratzell*

Welcoming of Guests ..... *Dr. J. P. Parker*

Laying of Wreaths on the Grave—

The University of North Carolina
The Wilson Family
The Elisha Mitchell Scientific Society
The Carolina Mountain Club
The Western North Carolina Historical Association
The Southern Appalachian Mineral Society
The Asheville Chamber of Commerce

Introduction of Speaker ..... *Chancellor Robert B. House*
The University of North Carolina at Chapel Hill

Principal Address ..... *Mr. D. Hiden Ramsey*

Music for this occasion was provided by a brass ensemble from the Brevard Music Center, through the courtesy of Dr. James C. Pfohl, Musical Director.

After the ceremony, the Asheville Chamber of Commerce gave a luncheon for the members of the official party who had been so notified. Meals and refreshments for other visitors were available as usual at the restaurants and stands on the mountain.

JOURNAL
OF THE
Elisha Mitchell Scientific Society

Volume 73 November 1957 No. 2

ELISHA MITCHELL AND MOUNT MITCHELL[1]

By D. Hiden Ramsey

On June 16, 1858, the body of Elisha Mitchell, Doctor of Divinity and late Professor of Chemistry, Mineralogy and Geology in the University of North Carolina, was buried on the summit of this mountain in perhaps the most impressive ceremonies ever held in Western North Carolina up to that time. Certainly, never before in the history of this section had so many people from such varied walks of life incurred such physical hardships in attending the burial service of any individual.

Elisha Mitchell was no eminent statesman, no military hero. He was not a native of this state, much less of this region. He was a college professor and a minister of the gospel. Many, perhaps most of those who attended the exercises in honor of this gentle man of truly prodigious learning were relatively unlearned mountaineers whose daily lives were largely restricted to the valleys and mountain slopes which they inhabited and to whom the University of North Carolina was little more than a name. For all that, this man was so lovable, his associations with these mountains were so intimate, the drama of his last days and the tragedy of his death were so moving that many people, great and humble, made the hard journey to this place—perhaps the most isolated burial spot in eastern America—to pay their respects to his memory.

When Dr. Mitchell's body was found on July 7, 1857, by Big Tom Wilson, it was brought the next day to this peak on the broad and willing shoulders of mountaineers. A casket had already been carried here from the Swannanoa side of the mountain. Those who had joined in the long and arduous search for the missing scientist felt keenly and argued strongly that the most appropriate resting place for the great and good man was here on the top of this mountain. They yielded only because they were told that the members of the Mitchell family, already reconciled to the worst, had requested that the body, when found, be brought to Asheville and buried in the Presbyterian Church cemetery.

The body was borne around the rugged slopes of what we now know as Clingman's Peak and Potato Knob and down the valleys of the North Fork and Swannanoa rivers to Asheville. There the body was buried. But the loyal and loving people of Buncombe and Yancey counties were not content. They continued to insist, individually and collectively, in private conversations and in a public meeting, that the body of Elisha Mitchell belonged here and nowhere else. Finally, the members of his family consented to the reinterment of his body on this

[1] Part of the principal address at the Memorial Service on Mount Mitchell, June 27th, 1957.

peak in whose "scientific exploration"—to borrow the apt words of his epitaph —he had lost his life.

The removal here was no simple undertaking. It was made possible only by the love and respect which the people of this mountain region cherished for Dr. Mitchell and by the cooperation of many strong arms and backs. On June 14, 1858, the body was exhumed. Early the next morning, the difficult task was begun. A full day and a half of almost unremitting labor were required. During the last third of the journey, the casket was borne on a sled drawn by oxen. At many difficult places, hardy mountaineers were compelled to shift the burden to their own shoulders. For the last several hundred yards, only surefooted men, capable of great physical exertion, were equal to the task.

On June 16, the ceremonies were held where we stand today. By horseback and by foot, the people came in large numbers over narrow and steep trails. Present were professors, trustees and students of the University. Out of the valleys trudged mountaineers of Yancey, Buncombe and McDowell counties. In keeping with the eternal fitness of things, one of their own number was chosen as chief marshal and they were accorded the first places in the funeral procession.

The speakers, Bishop James H. Otey of Tennessee and President David L. Swain of the University of North Carolina, spoke from a pulpit of upraised stones. As a young man, Bishop Otey had studied under Dr. Mitchell at the University, and he had come from the bedside of a sick relative in Mississippi to preach the funeral oration of his deeply beloved teacher. A mountainer by birth, President David L. Swain had achieved extraordinary distinction in public life before he went to the University as its President. He had always been a steadfast, tireless, and even vehement defender of Dr. Mitchell's right to be known as the first person who had truly measured the altitude of this peak.

Ninety and nine years later we come to this spot to commemorate with these simple exercises the centenary of the death of Elisha Mitchell. Appropriately enough, these ceremonies are under the auspices of the same University of North Carolina which he served with such fidelity and distinction. The long years that have come and gone since that far-off day have made it more famous without diminishing in the slightest its indebtedness to his hallowed memory.

This is not the place—nor am I the person—to undertake to measure the stature of Elisha Mitchell as a scientist. Happily, that has already been done with rich and spirited—and I may even say irrefutable—scholarship by Dr. Archibald Henderson.[2] Some comments a layman may be permitted to make without laying himself open to the charge of unpardonable presumption.

Elisha Mitchell lived and studied and taught in the misty dawn of the scientific era that amazes us with its fabulous achievements. Then the University of North Carolina was one of the few colleges in the young nation that even tried to offer any genuine instruction in the important sciences. Then the best of the laboratories were primitive by today's standards.

Throughout his active life, Elisha Mitchell had an unquenchable thirst for

[2] For a condensation of Dr. Henderson's account, *see* the succeeding article in this issue of the *Journal*.

knowledge, for all knowledge. He read deeply, traveled widely, observed keenly, spoke frequently and wrote copiously. The natural consequence was that at the time of his death, Dr. Mitchell possessed the widest range of information of any man in North Carolina and was in fact one of the most learned men in the nation. His knowledge of the state, its geography, its natural resources and phenomena was literally unrivaled. He was something more than a scholar stuffed gluttonously with all manner of miscellaneous and undigested learning. He was a great teacher. He had a positive genius for kindling the interests, winning the affections and informing and quickening the minds of his students. Long after he had fallen on sleep, many, many men in this and other states proudly boasted as one of their claims to good fortune: "I studied under Elisha Mitchell at Chapel Hill."

Withal and perhaps most important of all, he had at all times and under all conditions that humility which is the beginning of wisdom and which is always the hallmark of the authentic scholar. Vast knowledge never made proud his spirit. It never weakened in the slightest the simple and tranquil faith into which he had been born and which he wore like a golden garment throughout his life.

But it is not about Elisha Mitchell, the scientist and teacher, that I would speak altogether today. We of Western North Carolina have a reason, deep in our history, for being grateful to his memory. Since the first settlers came to this general region, the Black Mountains, bulking so massive and so tall against the horizon, had always attracted the admiration and wonder of people. Many persons had come to believe that some of these peaks were higher than Mount Washington in New Hampshire which was then accepted by geographers as the tallest peak east of the Mississippi. But the New England mountain had been measured repeatedly and mere impressions avail little against demonstrable facts . . . Dr. Mitchell decided to settle the question scientifically. In the summer of 1835 he visited a peak which was commonly regarded as the highest mountain by the people of this area and which his own examinations on the ground confirmed as the master peak. The instruments which he used were crude. He made the mistake of underestimating the altitude of Morganton which he assumed to be 200 feet less than it was. His basic calculations rested on this assumed altitude. Even with this wholly understandable error, he found a peak higher than Mount Washington. When these findings were reported to the world, the people of Western North Carolina were jubilant while the people of New England were dismayed, even outraged. Dr. Mitchell's own reputation as a scientist gradually won acceptance.

Returning in 1844 with more accurate instruments and with correct information about the true altitude of Morganton, Dr. Mitchell measured again the altitude of the peak which he had visited nine years before. This time he found the altitude to be 6672 feet or only twelve feet less than the figure officially established within the easy memory of the living, and now universally accepted. It should be added parenthetically that this measurement was the most accurate made until recent years.

Naturally, fame came to Dr. Mitchell, and the fame of one person often has the ugly consequence of arousing envy in others. The gentle, shy scientist soon

found himself involved in an unhappy, even rancorous controversy that darkened the last years of his life and that set in motion a train of events that led to his peculiarly lonely and tragic death on the northwestern slopes of this mountain. I have neither the time nor the disposition to recall the various chapters of the bitter dispute that broke around his head. In the little that I have to say today, I wish to be as charitable as I can to Thomas L. Clingman without being recreant to the truth or without being disloyal to the memory of the man whose nobility of character and glory of accomplishment we commemorate today.

Clingman was a man of ability, of unusual industry, of undoubted physical courage. He was later to serve as United States Senator and as an officer in the Confederate army. No one can fairly question his love of these mountains which he, born in another section of North Carolina, adopted as his own. But he had a relish for controversy that filled his none-too-serene life with successive disputes, that involved him in a famous duel, and that perhaps foreshadowed the mental breakdown which, sadly enough, he suffered in his last years. In 1855 he made a particularly unseemly attack on Dr. Mitchell. He claimed publicly that the scientist had never visited this peak and that the mountain which he was supposed to have measured was in fact another and more accessible peak in this range. With no little ostentation, Clingman visited this peak, measured it most inaccurately and then proclaimed that he was the first person to determine its correct altitude.

For such an unmannerly controversy, Dr. Mitchell was not well suited in temperament or in loose regard for the provable truth. His gentle spirit made him shrink instinctively from noisy disputes. He was not silent, however, under the charges leveled at him by this congenital disputant. He made several stern replies as the controversy dragged out over many contentious months. Finally he decided to return to the peak, to make new and more extensive measurements and to seek out familiar landmarks that would refresh his memories and support his claims. He arrived about the middle of June, 1857, and set immediately to work. On June 27, he decided to suspend temporarily his measurement activities and to go to the home of Big Tom Wilson on Cane River to check with him and other guides who had accompanied him on previous visits.

The story of Elisha Mitchell's death as it has been pieced together out of many facts and much speculation is too familiar to call for any recounting in detail on this occasion.[3] Suffice it to say that darkness overtook him and he fell to his death over a waterfall on Mitchell's Creek. When he failed to return to the appointed place at the appointed time, his son was deeply concerned. A party that went to Cane River brought back the disturbing information that he had not shown up at Big Tom Wilson's home. After a few more days of anxious waiting, the alarm was sounded and the search began in dead earnest. For four days, mountaineers combed the likely areas where he might have gone. Finally, his body was found by Big Tom Wilson.

[3] For previous accounts in this *Journal*, *see:* Charles Phillips, "A sketch of Elisha Mitchell," **1:** 9–18, 1884; Wm. B. Phillips, "The erection of the monument to Elisha Mitchell on Mitchell's High Peak," **5:** 55–59, 1888; and Kemp P. Battle, "Elisha Mitchell, D.D.," **30:** 157–164, 1915.

As we view matters from the vantage point of a century come and gone, certain facts appear to be incontestable:

1. Elisha Mitchell was the first person to measure with any pretense to scientific accuracy any peak in the Black Mountains. When he made his first calculations in 1835, Clingman was not even a resident of this section.

2. Elisha Mitchell was the first person to establish to the acceptance of geographers the supremely important fact that the highest peak east of the Mississippi was not Mount Washington in his native New England but a peak in the Black Mountains.

3. The measurement which he made in 1844 was amazingly accurate. We know today that it was more nearly correct than the figure which Arnold Guyot determined ten years later.

4. Dr. Mitchell's body was buried here ninety-nine years ago—not because he had in life expressed such a wish or because his family had requested his interment here—but solely because the mountain people insisted that it "belonged" here.

5. His name has been officially—and let us hope unchangeably—bestowed on this peak and not for light or transient reasons. Furthermore within sight of this mountain is a county that also bears his name.

The controversy in which he was involved through no provocation or wish of his own was never settled to the complete satisfaction of everyone. This much I would like to say personally: I have made some study of the dispute. I do not pretend to speak with conclusive authority. I am personally convinced by the weight of the evidence that Dr. Mitchell did visit and did measure this peak in 1835 and again in 1844.

Any speech on this occasion would be most inadequate if it did not emphasize the deep affection in which Elisha Mitchell was held by the undemonstrative but unfailingly loyal mountain people. On every occasion, they evidenced their love of him. In his very vivid and historic account of the search for Dr. Mitchell's body, Zebulon Baird Vance referred repeatedly to the sincere feeling of personal bereavement which the members of the searching party exhibited when the body was found. In his newspaper account of the reburial ceremonies, Richard H. Battle reported that there were no more genuine mourners at these solemn exercises than the people of Buncombe and Yancey counties. In 1877 Judge David Schenck heard Big Tom Wilson tell in his own simple but graphic words the story of the finding of the body. Even then—twenty years later—the rugged mountaineer was forced to halt his story as the tears rolled down his weather-beaten cheeks.

No one with the scantiest knowledge of this lordly mountain and its history can stand here today without thinking of others who have been associated with it. First of all, there was Big Tom Wilson, the prince of mountaineers, the greatest of all bear-hunters, the most famous of all mountain guides, an unpolished but genuine gentleman, the loyal friend of the living Elisha Mitchell and the unresting defender of the dead Elisha Mitchell. Then there was David L. Swain who was born in the Beaverdam valley of Buncombe County, who attended the University of North Carolina, who served long and efficiently as its President and

who was the first person to bestow the name of Mitchell on this peak. More lately was Locke Craig, "the Little Giant of the Mountains," also an alumnus of the University, to whom more than to any other person we owe the pleasing fact that the primeval forests on the summit of this peak were saved from the woodsman's axe and dedicated to the ownership and enjoyment of the people of North Carolina.

This burial plot is the property of the University of North Carolina. Dr. Mitchell's daughter, Miss Margaret Mitchell, acquired it in 1887 and deeded it to the University the following year. It should be added that when the family decided to erect a monument here, they turned the necessary funds over to the University and entrusted the institution with the tender and difficult task of selecting, transporting and erecting it.

I like to think that this plot here on the summit of the highest peak in eastern America is just as much a part of the University of North Carolina as the Old Well or Old East or Old West. The ownership springs not only from a legal title enforceable in a court of law. There is another covenant, more binding still. For

> "Here lies in the hope of a blessed resurrection the body of Rev. Elisha Mitchell, D.D., who, after being for 39 years a professor in the University of North Carolina, lost his life in the scientific exploration of this mountain in the 64th year of his age, June 27th, 1857."

ELISHA MITCHELL: SCIENTIST AND MAN*

By Archibald Henderson

Elisha Mitchell, the eldest son of Abner Mitchell, a prosperous farmer, and his wife, Phoebe Eliot, was born in Washington, Litchfield County, Connecticut, August 19, 1793. Elisha's mother was a descendant in the fifth generation of the celebrated John Eliot, "Apostle to the Indians." Elisha's ancestor, the Rev. Jared Eliot, M.D., D.D., was distinguished for versatility, being famous in his day, not only as orthodox theologian, but for his comprehensive knowledge of history, natural philosophy, botany, and mineralogy. He was a correspondent of Benjamin Franklin and Bishop Berkeley, and in 1762 was honored by the Royal Society of London with a gold medal for a valuable discovery in the manufacture of iron.

* Part of the address delivered at the Centennial Memorial Meeting of the Elisha Mitchell Scientific Society, May 14, 1957, at the Carolina Inn, Chapel Hill. In acknowledging with sincere appreciation the honor conferred upon him in being chosen to deliver this commemorative address, the speaker mentioned that this was the fourth time he had spoken by invitation before the Society. The other occasions were as follows: "The Elisha Mitchell Scientific Society: Its History and Achievements," in commemoration of the fiftieth anniversary of the founding of the Society, November 14, 1933; "The Elisha Mitchell Scientific Society: Retrospect and Prospect," 400th meeting, April 9, 1940; and "Science and Art: Some Sparks from the Forge of Life," 500th meeting, December 9, 1952.

As a child, Elisha was extraordinarily precocious; and it is of record that, at the age of four, he delighted his elders by an admirable display of sangfroid at a school exhibition. From an early age he reveled in wide and varied reading and enjoyed nothing so much, when a small lad, as retailing, to his open-mouthed and wide-eyed schoolfellows, the gist of his findings and the significance of the illustrations. He attended the classical school at Bethlem, Litchfield County, of the Rev. Azel Backus, who later became president of Hamilton College, New York.

Elisha Mitchell was graduated from Yale College in 1813. He seems to have taken as his model, from among his preceptors, Benjamin Silliman, professor of chemistry and mineralogy, whose true *métier* was realized in founding *The American Journal of Science and Arts*; and as he was its sole editor for twenty years (1818–1838), it came to be universally termed *Silliman's Journal*.

After graduation, Elisha Mitchell first taught in a school for boys at Jamaica on Long Island; and in 1815 became the headmaster of a school for girls in New London, Connecticut. In 1816 he became a Tutor at his alma mater, where he attracted the attention of faculty and students alike by his skill in the teaching of mathematics. When Joseph Caldwell was elected president of the University of North Carolina, Mitchell, aged twenty-four, succeeded him in the chair of mathematics; and Denison Olmsted, his classmate at Yale, was given the newly created chair of chemistry and mineralogy. The elevation of scholarly standards at Chapel Hill by the irruption of these "barbarians from the North," was jocularly noted by Dr. William Hooper, head of the classics department.

Mitchell made the long and tiresome trip from Washington, Connecticut, to Chapel Hill, North Carolina, in a gig, a sort of one-seat, two-wheel sulky. He became engaged to be married to a young girl he had met in his girls' school in New London, Mary Sybil North, daughter of an eminent physician there. She seems to have entertained some doubts as to the durability of romantic love after marriage; and to relieve her doubts, Elisha wrote her a letter from Chapel Hill, giving a conspicuous example of marital fidelity and unwavering devotion. This was the family of a genial gentleman, Major Pleasant Henderson, former University Steward and a member of the North Carolina Assembly, who lived where the United States Post Office now stands.[1] Mitchell as a bachelor roomed at Mrs. Nunn's "House of Entertainment" located just south of the present Baptist Church; and boarded with the Henderson family. After his marriage, Mitchell took as his permanent home the first President's House, where the Communications Center now stands; and this the Mitchell family occupied until 1857. Elisha wrote to his Mary in February, 1818, concerning this Major Pleasant Henderson, the old Revolutionary veteran, and his wife Sarah, niece of Governor Alexander Martin. They had then been married for thirty-two years, she fifty-four, he sixty-three years of age. "You can hardly imagine how much he and his wife love each other," reports Elisha. This seems to have proved the clincher; for Elisha returned to New London in 1820 and brought to Chapel Hill

[1] The street running north past the Post Office was named for him.

his bride, definitively persuaded by this example of the persistence of true love after matrimony.[2]

Elisha Mitchell had a very definite philosophy of education, which he set forth in occasional pamphlets and newspaper articles. Most conspicuous was his conviction that women from early years should be as highly trained as men in all branches of knowledge. He was far ahead of his time in his views on the education of women.[3] So diverse were Dr. Mitchell's activities, in connection with the University and the town of Chapel Hill, over a period of thirty-nine years, that the University's historian, the late Dr. Kemp P. Battle, described him as possessed of a soul that "thirsted for all work, as well as for all knowledge." From the time of his arrival here he acted informally as Bursar, and in 1837 he was officially appointed to this post, with broad powers of supervision over buildings and grounds. He exercised his restless energy as amateur landscape architect and road builder; and, hailing from New England where "stun fences," as they were called in the Connecticut vernacular (our rock walls) were a striking feature of the landscape, he persuaded President Swain, by building a rod of model stone fence, to commission him to direct the construction of a rock wall entirely around the enlarged campus. He took a lively interest in everything affecting the town, its development and government.

As botanist, mineralogist and geologist he visited almost all sections of North Carolina, and his colleague, Dr. Charles Phillips, says of him that he was more intimately informed than anyone else in North Carolina about her "mountains, valleys, and plains, trees, flowers, vines and mosses, rocks, stones, sands, clays and marls, fishes, shells, beasts, birds, and—bugs." He had a magnificent library in both science and general literature.[4] Because of the variety of his interests and occupations, we may think of him as a mature "Admirable Crichton." A catalogue of Mitchell's skills and positions almost defies credulity: mathematician, surveyor, road builder, engineer, agriculturist, botanist, geologist, mineralogist, bursar, overseer of buildings and grounds, professor, acting president, preacher, town commissioner, and magistrate of police. It may well be queried whether the "chair" he occupied here was a sofa, a davenport, or a settee.

In the class room, Mitchell enthralled his students by the range of his erudition and the lucidity of his exposition. Whenever any puzzling or cryptic question arose on the campus, the querist was always advised: "Ask Dr. Mitchell." He

[2] A copy of this letter was supplied me by a granddaughter of Elisha Mitchell, Mrs. Hope Summerell Chamberlain, long a resident of Chapel Hill. I am indebted to her for the opportunity to read her very interesting unpublished biography of Elisha Mitchell, written primarily for his descendants. A typescript copy is on file in the Louis R. Wilson Library here.

[3] At this point the speaker read from an original letter presented by Dr. J. Eliot Coit, of Vista, California, to the Elisha Mitchell Papers in the Southern Historical Collection. In this letter to his wife (July 22, 1822) Elisha Mitchell promises a reward of Bechtler gold dollars to the three girls "if they study Arithmetic in my absence *very well*."

[4] This library fell short of the holdings of the University library by only three volumes. In 1857 when his library was sold to the University of North Carolina, a 15-page printed catalogue, of great historical interest, was issued. A copy is preserved in the Louis R. Wilson Library.

had a photostatic memory and a photographic brain. On one occasion when he was scheduled to deliver a lecture before the North Carolina Agricultural Society, a friend exclaimed: "I'll wager Dr. Mitchell begins at the Garden of Eden." And, sure enough, he did—beginning in the Garden of Eden, passing through Egypt and Canaan, Greece, Rome, and Great Britain, and cheerfully ending his journey in the adjoining county of Chatham!

It is not easy to assay Mitchell's position as a scientist, as his interests were diffused so widely, and in consequence his vast and heterogeneous knowledge was spread so thin. He was indefatigable in his search for facts, but he was not a devotee of research, as we understand and practice it to-day: the co-ordination of facts and discoveries and the development therefrom of new scientific ideas and theories. He was called to the chair of mathematics here, but he was not a mathematician, publishing nothing on the subject and making no contributions to the science. He was, however, as astronomer, expert surveyor, and engineer, skilled in the use of various scientific instruments: the level, the quadrant, the compass, the theodolite, the barometer, and the telescope. To facilitate the tasks of teaching, he had printed a useful handbook in natural history, a manual of chemistry, and a short textbook on geology, including in particular the geology of North Carolina, accompanied by a geologic map. As his admired teacher at Yale, Benjamin Silliman, was the editor of *The American Journal of Science and Arts*, Mitchell published many of the results of his geographical, physical, geophysical, geological, and mineralogical findings in that journal over a period of years. The more popular subjects he discussed at length in magazines and newspapers such as the *New York Albion*, the *Raleigh Register*, the *Asheville Spectator*, and *The Harbinger* of Chapel Hill. In attempting an estimate of Mitchell as a scientist, it must be borne in mind that young men like Mitchell and Olmsted grew up along with these youthful sciences which were developing as these youthful scientists were teaching them. Considering the enormous time and energy devoted to his non-scientific activities, we are forced to conclude that Mitchell was not an unproductive scholar, on the basis of the number, if not in all cases the permanent value, of his scientific papers, pamphlets, and books. Had Mitchell published only his contributions to the knowledge of the geography and geology of North Carolina, and the descriptions and heights of its lofty mountain peaks which he explored and for the first time scientifically measured, he would still hold a distinctive place in the history of American science.

On December 31, 1821, following its establishment two years earlier, Olmsted wrote to the State Board of Internal Improvement proposing the authorization of a geological survey of North Carolina. After considerable labor in the field with competent assistants, he issued his *Report on the Geology of North Carolina Conducted under the Direction of The State Board of Agriculture* (Raleigh, N. C.) in two installments, Part I dated November, 1824, and Part II, November, 1825. When Olmsted was called to Yale in 1825, Elisha Mitchell succeeded not only to his chair of chemistry, geology and mineralogy, but also to the task of completing the geological survey of North Carolina. In 1827 he published Part III of the survey, and in 1829 he completed, as Part IV, a supplementary

report. [At this point the speaker exhibited copies of these publications.] These four parts constitute a scientific reconnaissance of the state's geophysical features and mineral resources. Olmsted devoted his attention primarily to listing the minerals of economic importance; but Mitchell gave more attention to theories regarding the age and origin of certain geophysical formations. The claim has been made that "the first geological survey completed at state expense was that of Massachusetts, in 1830–1833, by Edward Hitchcock."[5] Indubitably the first geological survey completed at state expense in the United States was that of North Carolina, 1825–1829, by Denison Olmsted and Elisha Mitchell.[6]

In the course of his tours throughout North Carolina, Mitchell became particularly interested in western North Carolina and the large number of lofty peaks there. As a botanist, he was familiar with the works of the famous botanists, André Michaux and his son, François André Michaux, who had made a number of tours through Western North Carolina, from 1789 to 1802, and been profoundly impressed with the heights of the peaks in that region. It is said that in June, 1789, the elder Michaux climbed the "Yellow Mountain" (unidentified), jubilantly sang *The Marseillaise*, and expressed the extravagant conviction that this was "the highest peak on the American continent." His son, who visited these mountains in 1802, came to the more moderate conclusion that here were to be found the peaks of greatest elevation in the entire nine hundred mile stretch of the Alleghenies. The peaks he mentioned, in descending order of elevation, according to his erroneous guess, were: Grandfather, Iron, Yellow, Black, and Table.[7] The reason for the belief of the French botanists was that they found plants on these mountains which were not encountered again until the lofty Canadian peaks were reached.

In 1825 John C. Calhoun stated to David L. Swain that in Buncombe County were to be found the highest mountains on the American continent east of the Rocky Mountains, since here were located the headsprings of the longest rivers in the United States, east of the Rockies. These rivers found their way in opposite directions to the Atlantic Ocean and to the Gulf of Mexico via the Mississippi River; and in consequence the sources must be located at the points of greatest elevation.

Mitchell was especially fascinated by the "great Black Mountain" which he described as a "long curved ridge, 15 or 20 miles in length, its base having some-

[5] See H. L. Fairchild, "The Development of Geologic Science," *Scientific Monthly* **XIX** (1942), 77–101. This claim is patently incorrect, as North Carolina's survey was begun more than five years earlier, and completed four years earlier, than that of Massachusetts. The reference to Fairchild's article was kindly furnished me by Albert N. Bove, Geologist in Charge, Geologic Map Records, U. S. Geological Survey, Department of the Interior, Washington, D. C.

[6] For further information on this subject, consult Archibald Henderson, *The Old North State, and the New*, two vols. (1941), II, 721–722.

[7] François André Michaux's experiences are recorded in his *Journey to the West of the Allegheny Mountains* (French edition, 1804, English translation, 1805). In the second English edition (1806), pp. 259–260, are listed the five mountains mentioned. Obviously the Yellow Mountain is not Grandfather Mountain, as has been sometimes stated, since both Yellow and Grandfather are included in the list.

what the form of a common fishhook." He recorded his conviction in the following words: "The Black is in my opinion the highest land of the whole eastern mountain system, from the White Mountains to the Gulf"; and expressed his intention of investigating "these lofty summits." Faced by the silent challenge of this formidable mountain bastion, Mitchell eventually picked up the gauntlet and devoted the most arduous and painstaking efforts during the last twenty-two years of his life to climbing and surveying the high peaks and determining their altitudes, a task which had never thitherto been accomplished, or even attempted.

The fascinating story of the prolonged controversy over the measuring and naming of the peak now known as Mount Mitchell, I shall envision in the form of a five-act drama, which means conflict, with its successive climaxes and tragic ending. The finale is personal disaster, but the ineluctable triumph of science.

The first act of the drama opens in the summer of 1835 when the hero, whom we shall designate the Scientist, measures the mountain which we shall call Peak A. He learned, on inquiry of the Yancey County mountaineers, which peak was generally accepted as the highest on the Black Mountain ridge. Ascending then to the summit of Yeates's Knob, from which he obtained a panoramic view of the mighty ridge, he determined, by the use of quadrant and compass, the highest peak and also its compass bearing. Then, directed by the most expert guides in that neighborhood, he ascended this highest peak, observed with his quadrant that it was appreciably higher than any of the other visible peaks, and took the barometric measurement of the elevation above sea level. Deliberately assuming a very conservative figure for the height of Morganton, since barometric measurements of low level (say 1,000 to 1,500 feet) are notoriously inaccurate, he arrived at a disappointing figure for the height of the peak he had just measured, which he had surmised would be at least 100 feet higher than the highest peak in the White Mountains of New Hampshire. His figures showed that Black was higher than White, Peak A loftier by 48 feet than Mount Washington, thitherto credited with being the highest peak on the American Continent east of the Rocky Mountains. This announcement created a great sensation in North Carolina, where it was hailed with delight, and in New England, where it was greeted with skepticism, not to say indignation. The news quickly spread throughout the nation, and the hero acquired his first taste of fame.

The second act opens in 1844 with a new attempt at measuring Peak A, which the Scientist thought would have registered much higher had his instruments been more accurate. This time he was equipped with the best type of barometer to be found in the world, manufactured by the great French scientist, Gay-Lussac, which Mitchell's wife's brother, Harvey North, had procured for him in Paris. The final figure he found in 1844 for the height of Peak A was 6,672 feet, almost 250 feet above Mount Washington, an excess about five times the amount of the excess in the doubtful survey of 1835. Hercules had successfully accomplished his Second Labor, and won fresh and louder acclaim.

The third act begins with the entry of the villain of the piece, whom we shall designate the Politician. He hailed from the mountain region, was in part of Indian extraction, and had been one of Mitchell's students, graduating in 1832. He was arrogant, opinionated, and insolent; and was cursed with the deadly

malady of *hubris*, overweening pride, which brought the downfall of many an antagonist and protagonist in Greek tragedy. Inordinately proud of the great mountain ranges, the Blue Ridge and the Great Smokies, he felt that he had a proprietary right in them; and the jocular Zeb Vance remarked that there are some Yancey County mountaineers who believe that the Politician "superintended the creation of those mountains and therefore has a right to know more about them than anyone else."

In 1839 was issued Smith's *Geography and Atlas*, which was widely popular.[8] When the agent applied to President Swain for suggestions as to revision, Swain, an enthusiast over Mitchell's achievement in bringing national recognition to North Carolina, supplied him with the following ill-advised and incorrect statement, which appeared in the printed work: "Mount Mitchell in this state has been ascertained to be the highest point of land in the United States, east of the Rocky Mountains."[9] The seeds of incipient dementia had probably already been sown in the Politician's brain, as the mental alienist might well divine from the actions, conversations, correspondence, and published articles and pamphlets he issued. Inspired by delusions of grandeur, he made many trips throughout the Black Mountain region, conversed with many of the mountaineers, and openly charged that Peak A which Mitchell had measured was in Buncombe County, and that the highest peak was several miles away across the dividing line in Yancey County. He further maintained that Mitchell was ignorant of the terrain, not knowing one peak from another, and that the expert guides were inexpert or venal or prepossessed in Mitchell's favor.[10] *Hubris*, the tragic fault, was already in evidence, and the obsession of rancor and jealousy and the *idée fixe* of egoism were already clearly manifest.

The fourth act validates the Scientist's surmise that the experiment of 1835, which was based upon an approximate value of the height of Morganton, would prove to be a gross underestimate. Engineers of the Louisville, Nashville, and Charleston Railroad, by the more accurate method of the level, later determined the height of the Morganton railroad station to be 1,169 feet above sea level.[11] Mitchell's height for Morganton (968 feet) was taken by barometer at the public square, which was 31 feet higher than the railroad station. Therefore the corrected height for Mitchell's Peak A was 1,169 + 31 + 6,476 − 968, namely 6,708 feet. This figure proved to be in spectacularly accurate agreement with the later measurement of Mt. Mitchell, 6,701 feet, made in 1856 by Arnold Guyot, Swiss scientist, then Professor of History and Engineering at Princeton, using the excellent barometer manufactured by Ernst of Paris.[12]

[8] Roswell C. Smith, *Geography and Atlas* (New York: Cody and Burgess, 1839).

[9] There are peaks in South Dakota and Texas which are far loftier than any of the peaks east of them on the American continent. Harvey Peak in South Dakota is 7,242 feet high; and Guadalupe Peak, in Texas, is 8,751 feet high. Letter to the writer (May 3, 1957) from Albert N. Bove. See footnote 5, above.

[10] See "Topography of Black Mountain," *Tenth Annual Report of the Board of Regents of the Smithsonian Institution* (1858).

[11] See first report of the President and Directors of the Louisville, Nashville, and Charleston Railroad (1837).

[12] See "Guyot's Measurements of the Mountains of Western North Carolina," *Asheville*

In September, 1857, James W. Wilson, under the direction of Major J. C. Turner, Chief Engineer of the Western North Carolina Railroad, using levels, found the height of Mount Mitchell to be 6,711 feet. Phenomenal! Mitchell in 1835—6,708, Guyot in 1856—6,701, Wilson in 1857—6,711. Here we have an impressive scientific demonstration that all three men were extraordinarily accurate engineers. As Mitchell's accuracy in the barometric measurement of other peaks was never questioned, the conclusion is scientifically inescapable: Peak A was none other than Mount Mitchell.

The height of Mount Mitchell taken by the United States Geological Survey by the most refined methods was: 6,688 in 1930, 6,684 in 1950. The mean of these two figures is 6,686. The height of Mount Mitchell taken by Elisha Mitchell was 6,708 in 1835, 6,672 in 1844. The mean of these two figures is 6,690. The difference between these two means is only 4 feet or about six hundredths of one per cent! It is melancholy to reflect that Mitchell's expedition, with its tragic ending, in 1857, designed for final refutation of the insolent charges of a scientific ignoramus, was superfluous. Mitchell's case had already been proved.

The fifth act opens in 1855 with the villain of the piece, the Politician, moving in for the kill. With great fanfare, on September 8, 1955, the Politician measures the altitude of the highest Black Mountain peak (present Mount Mitchell) and reports it to be 6,941 feet—257 feet loftier than its true height. Preposterous! The villain was foiled. He had reduced himself to absurdity.

Stung by the villain's charges, the apparent endorsement of the villain's arguments by Professor Joseph Henry, Secretary of the Smithsonian Institution, and the final insult, the attachment of the villain's name, in William D. Cooke's map of North Carolina,[13] to the peak which for eighteen years in Smith's *Geography* had been designated "Mitchell's Peak, highest east of the Rocky Mountains," the Scientist prepared to ascend this peak, for the third time, and measure its altitude. He used two Green's barometers, which were recommended by the Smithsonian Institution. Accompanied by son Charles, daughter Margaret, and a colored boy, he began the ascent, reaching the Mountain House on June 27, 1857.[14] Leaving the others, he set off to visit the home of the elderly William Wilson, and to obtain attested accounts from him and others of their former visits to the mountain as his guides. He never returned alive: his body, in a clear pool below a waterfall, was found by "Big Tom" Wilson, on July 7, after a search by hundreds of people lasting eight days.[15] The scientist's watch recorded pre-

News, July 18, 1860. This paper is reproduced in F. A. Sondley, *A History of Buncombe County*, North Carolina (1930), II, 785-797.

[13] This map was published early in 1857.

[14] The original of Elisha Mitchell's presumably last letter, addressed to his wife, is headed: "Patton's Mountain House, Saturday Morning, some day in June, 1857." It is preserved in the Southern Historical Collection.

[15] In a letter from Chapel Hill, N. C. (July 11, 1857), commenting on Mitchell's death, Tobias Gibson, a student, writes to his father, Tobias Gibson:

P. S. July 12th, 1857

I do not know who can fill his place, either as an instructor in the University or as a man in the community—When such men leave the world society receives a shock—Although the most rigid man in the Faculty he was the most active—he was engaged with his recitations

sumably the instant of his death: 18 minutes past 8 o'clock. The moving hands stop! The scientist belongs with the immortals. A North Carolina county (1861) and a great mountain are named for him; three successive monuments have marked his grave during the past century.[16] His remains lie on the highest spot of the Elisha Mitchell State Park.[17]

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longer, and he studied harder to impart that knowledge which was beyond the reach of the students than all the rest of the board together—He was beloved by every student in College—

This letter is preserved in the Gibson-Humphrey Papers, Southern Historical Collection.

[16] The triangulation marker, placed by the United States Coast and Geodetic Survey on Mount Mitchell in 1876, first officially gave it that name. The latest official determination of the height of Mt. Mitchell (6,684 feet) is recorded in a bulletin of the United States Geological Survey, entitled "Heights and Mean Altitudes in the United States, Its Territories and Possessions" (1950). For much important data on the names and heights of high peaks, and an impressive photographic panorama of the Black Mountains, I am indebted to the courtesy of H. Arnold Karo, Rear Admiral, USC&GS, Director, Washington, D. C. (letter of date April 26, 1957).

[17] The man whom I have termed the "Politician" and even the "villain" in this epitomized drama was Thomas Lanier Clingman, born in Huntsville, North Carolina, July 27, 1812. He served as both a Member of the National Congress and as United States Senator. At the outbreak of the Civil War he resigned from the Senate and joined the armies of the Confederacy, became a brigadier general, and surrendered with Johnston's army at the Bennett House, near Durham. He died in an asylum for the insane at Morganton, North Carolina, November 3, 1897.

In recognition of his indefatigable labors in studying the mountains of Western North Carolina, publicizing their lofty majesty and spectacular beauty, and measuring the heights of some of the peaks, two of them were given his name: the third highest of the Black Mountain ridge (6,567 feet) and the highest of the Great Smoky Mountains (6,642 feet).

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

1956–57

530TH MEETING, OCTOBER 9, 1957

W. A. BOWERS (Physics): *Inelastic Scattering of Neutrons from Crystals.*

G. R. MACCARTHY (Geology): *Something about Permafrost.*

Forty-three associate members were elected. Seven full members were elected, as follows: Walter R. Benson (Pathology); Sigurdur Jonsson, Earl T. Brown (Pharmacy); Lillian M. Youngs (Zoology); W. F. Little, M. Ikehare (Chemistry); David L. Mitchell (Anatomy). President Engels announced that future business meetings would take up (1) the question of the Coker Award and (2) the hundredth anniversary of the death of Elisha Mitchell.

531ST MEETING, NOVEMBER 13, 1956

J. L. IRVIN, JEAN ROTHERHAM, E. M. IRVIN AND D. J. HOLBROOK (Biochemistry): *The Synthesis of Proteins and Nucleic Acids in the Nuclei of Liver and Hepatoma Cells.*

J. C. MORROW (Chemistry): *Low Temperature Therms and Magnetic Properties of an Iron-Manganese Alloy.*

Sixteen associate members were elected; also two full members, C. R. Bell and H. Ahles (Botany).

532ND MEETING, DECEMBER 11, 1956

VIRGIL I. MANN (Geology): *Identification of Kidney Stones with a Petrographic Microscope.*

HAROLD HOTELLING (Statistics): *Relations of Factor Analysis to the Newer Multivariate Statistical Methods.*

533RD MEETING, JANUARY 8, 1957

W. R. MANN (Mathematics): *Error Growth in Numerical Approximations.*

M. L. GRANSTROM (Sanitary Engineering): *The Disproportionation of Monochloramine.*

534TH MEETING, FEBRUARY 12, 1957

DAVID L. MITCHELL (Anatomy): *Some Aspects of the Sexual Dimorphism of the Salivary Glands of the Mouse.*

J. F. DASHIELL (Psychology): *Fatigue-Induced Monocular Diplopia: Second Report.*

535TH MEETING, MARCH 12, 1957

C. N. REILLEY (Chemistry): *Metal Analysis with Complexones.*

WALTER WHEELER (Geology): *Fossil Brains.*

Dr. Roe reported on the plans for the commemoration of the hundredth anniversary of the death of Elisha Mitchell, which were outlined in a letter to Chancellor House.

Dr. Hooker read the recommendations of the special committee on the Coker Award: each department should act as a screening committee by nominating only a distinguished thesis, not merely the best in that year, and should submit a critical evaluation of it that will aid the judging committee in reaching its decision; in the absence of an outstanding thesis, the Award would not be given in that year. The recommendations were unanimously approved by a formal vote.

President Engels recommended that the annual business meeting be conducted at the April meeting, in view of the special ceremonies planned for May. This recommendation was unanimously approved.

536TH MEETING, APRIL 9, 1957

W. J. KOCH (Botany): *Structure of the Swimming Spores of Aquatic Fungi.*

E. R. LONG (Psychology): *Preliminary Observations of Operant Conditioning in Children.*

The Treasurer's report was read, and the Auditing Committee (C. D. Beers, S. Claiborne Jones) reported the books in order.

The Nominating Committee (J. E. Adams, W. C. George, and P. E. Shearin)

presented the following nominees for 1957–8:

President—C. W. Hooker
Vice-President—J. C. Morrow
Secretary-Treasurer—H. O. Thompson

The slate was unanimously elected.

537TH MEETING, MAY 14, 1957

As part of the centennial exercises commemorating the death of Elisha Mitchell, a dinner sponsored by the University was held at 7 p.m. in the Carolina Inn ballroom. It was attended by members of the Elisha Mitchell Scientific Society and special guests, and was presided over by President W. L. Engels. The centennial program was introduced by Dr. Arthur Roe, Program Director. Dr. E. D. Palmatier, Chairman of the Department of Physics, exhibited some early scientific instruments which had been obtained largely through the influence of Dr. Elisha Mitchell. Chancellor R. B. House then introduced the speaker, Dr. Archibald Henderson, who gave a scholarly talk on Dr. Mitchell's history and achievements, and his contribution to the University, the State, and science in general. A condensation of part of this address is printed elsewhere in this issue of the *Journal*.

HERMAN O. THOMPSON, *incoming Sec.-Treas.*
for HAROLD G. MCCURDY, *outgoing Sec.-Treas.*

PROCEEDINGS OF THE FIFTY-FOURTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

The fifty-fourth annual meeting of the North Carolina Academy of Science was held at Wake Forest College, Winston-Salem, on May 3 and 4, 1957.

The Academy met in its first session at 10:30 a.m. on May 3 with President Harkema presiding. A general session of three invited papers was held on this occasion.

The business meeting of the Academy was held at 4:30 p.m. on May 3, at which time the following business was transacted:

The minutes of the 1956 meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society **72:** 167–201.

REPORT OF THE EXECUTIVE COMMITTEE

The Executive Committee met at the Science Building, Wake Forest College, on May 2 at 7:30 p.m. At this meeting the following matters were considered:

1. All persons applying for membership in the Academy by submitting blanks and dues to the Secretary-Treasurer were elected by the Committee. Names appear later in these Proceedings.
2. The participation of the Academy in the special issues of the Elisha Mitchell Journal in 1957 honoring Dr. Elisha Mitchell was approved.

3. The Committee recommended two persons for life membership, A. F. Thiel of Woman's College, U. N. C., and F. W. Sherwood of North Carolina State College.
4. The Secretary-Treasurer was asked to mimeograph a list of the members for 1957 and distribute to all the membership.
5. Voted to proceed with plans to secure a fidelity bond for the Secretary-Treasurer with coverage of $2000 at an annual premium of $5.
6. Approved the development of a High School Teaching Section of the Academy. The Committee expressed the hope that such a Section would be organized at this 1957 meeting.
7. Approved a travel allotment for the Secretary-Treasurer for the year 1957–58 not to exceed $100 with mileage at seven cents per mile.
8. Approved the development of a small committee to seek actively sustaining members for the Academy. The Committee asked Dr. Harkema, retiring president, and the Secretary-Treasurer to find one or more persons who would consent to work on such a project.
9. Voted to ask the lecturers who visit college science clubs under the leadership of the Collegiate Academy, to investigate the interest in such colleges in research and project work for their undergraduates and to encourage the staffs of such colleges to make known to the Collegiate Academy sponsors any needs they have for funds to carry on undergraduate project work.
10. Approved a plan for the Physics Section whereby up to twenty high school physics teachers, if recommended by members of that Section, may be extended honorary Academy membership for one year.
11. Approved in general a plan to begin work toward the writing of a history of science for North Carolina. The Committee suggested that the Elisha Mitchell Society be contacted with a view to obtaining their help in this work. The Secretary-Treasurer and the President of the Academy were directed to seek out Academy members who may be interested in active participation in this project. No financial obligation for the Academy is contemplated at this time.
12. The Committee recommended that the Academy accept the invitation of Duke University to meet on its campus in Durham next year, 1958, at a date to be determined by the new Executive Committee.
13. The following were elected to membership in the Academy or reinstated to active membership:

Adcock, Louis Henry, Wilmington College, Wilmington
Anderton, Miss Laura G., Woman's College, U. N. C., Greensboro
Andrew, Murray M., Siler City
Bishop, Bruce B., Hickory
Brannock, Dr. Ned Francette, Elon College
Brown, Mrs. W. H., Shelby
Carpenter, Dr. David Williams, Duke University, Durham
Carnes, William Capers, Jr., N. C. State College, Raleigh
Clark, Mrs. Frances C., Rich Square
Clarke, William M., Duke University, Durham

Cooke, Mrs. Mildred Watkins, Apex
Coon, S. L., Fallston
Crawford, Eugene C., Jr., Duke University, Durham
Cuyler, R. Duncan, Durham
Denning, Martin, Harrells
De Poe, Charles Edward, N. C. State College, Raleigh
Dodson, C. F., Western Carolina College, Cullowhee
Everett, Dr. Grover Woodrow, East Carolina College, Greenville
Everhart, Mrs. Kate C., Greensboro
Farlowe, Vivian, Meredith College, Raleigh
Fehon, Dr. Jack H., Queens College, Charlotte
Felton, Marguerite Norman, Woman's College, U. N. C., Greensboro
Fisher, Mrs. Mary Janes, Concord
Foreman, Dr. Charles W., Pfeiffer College, Misenheimer
Fowler, Dr. Ira, University of N. C., Chapel Hill
Garrett, T. Rawdon, Ahoskie
Gay, R. L., Wake Forest College, Winston-Salem
Gordy, Dr. Walter, Duke University, Durham
Haesloop, John G., University of N. C., Chapel Hill
Hamrick, Dr. Phillip J., Wake Forest College, Winston-Salem
Hayes, Nelson A., Badin
Haynes, Dr. Hubert Calvin, East Carolina College, Greenville
Hill, Dr. J. N., Murphy
Hilliard, Roy C., Liggett & Myers, Durham
Hinkle, Dr. Robert O., Tulsa, Oklahoma
Hughes, Gilbert C., III, Florida State University, Tallahassee
LaChance, Leo Emery, N. C. State College, Raleigh
Leisner, Robert Stanley, University of N. C., Chapel Hill
Lewis, Dr. Lillian B., Winston-Salem Teacher's College, Winston-Salem
Livingstone, Dr. Daniel, Duke University, Durham
Lofland, Dr. Hugh B., Jr., Bowman Gray School of Medicine, Winston-Salem
McCracken, Hubert J., Oak City
McCreight, Dr. Charles E., Bowman Gray School of Medicine, Winston-Salem
McLane, S. Brooks, Jr., Davidson College, Davidson
McMain, Albert T., Jr., N. C. State College, Raleigh
McNeill, Dr. John J., N. C. State College, Raleigh
Means, Richard E., Liggett & Myers, Durham
Menius, Dr. A. C., Jr., N. C. State College, Raleigh
Moore, Z. Camanade, Fayetteville
Moser, Ruth Fain, Charlotte
Parker, Charles F., Wagram
Pearson, Miss Beatrice, North Wilkesboro
Pellicciaro, Dr. Edward Joseph, University of Delaware, Newark, Del.
Philippe, Dr. Roger J., Liggett & Myers, Durham
Phillips, Mrs. Sue Sherrill, Cameron
Potter, Mrs. James M., Raleigh
Prince, Capt. E. R., Jr., Air Force Academy, Denver, Colorado
Ramsey, Dr. Harold A., N. C. State College, Raleigh
Register, Thomas E., University of N. C., Chapel Hill
Reynolds, Dr. Thomas D., Duke University, Durham
Robertson, Dr. Clyde H., Jr., Pfeiffer College, Misenheimer
Saieed, Alfred Earl, Greenville
Sawyer, Dr. John Wesley, Wake Forest College, Winston-Salem

Sherwood, William Allen, Mars Hill College, Mars Hill
Shipp, William Beasley, Liggett & Myers, Durham
Shore, Mrs. Nancy B., Raleigh
Smith, William R., Jr., Wilmington
Snyder, Dr. Karl D., Montreat College, Montreat
Squibb, Dr. S. Dexter, Western Carolina College, Cullowhee
Stephenson, John Whitaker, Jr., N. C. State College, Raleigh
Strickland, Dr. J. C., University of Richmond, Richmond, Virginia
Sulkin, Dr. Norman M., Bowman Gray School of Medicine, Winston-Salem
Tait, Warren R., Atlantic Christian College, Wilson
Troyer, Dr. James R., N. C. State College, Raleigh
Ward, Thomas M., N. C. State College, Raleigh
Westbrook, Robert B., Statesville
Whisonant, Mrs. Eunice M., Greensboro
Wolff, Dr. William A., Bowman Gray School of Medicine, Winston-Salem

The above reports and actions of the Committee were approved by the Academy in its business session on May 3, 1957.

TREASURER'S REPORT

Financial Statement as of July 1, 1957

Receipts—Academy proper	
Checking account balance from July 1, 1956 (Security National Bank, Raleigh)	$309.90
Dues from memberships	1452.00
Research fund from A.A.A.S.	25.00
Collegiate Academy gifts for *Cancas* (Sigma Xi chapters at Duke University, N. C. State College and University of North Carolina)	125.00
Collegiate Academy funds deposited	27.68
N. C. Psychological Association (for copies of annual program)	6.00
Derieux Prize from Mrs. J. B. Derieux	50.00
Exhibit fees from commercial exhibitors (annual meeting)	40.00
A.A.A.S. for expense to Chicago meeting for Junior Academies	125.00
Total Receipts for Academy proper	2160.58
Receipts—Science Fair Program	
Entry Fees—from seven district fairs	1340.00
Medical Society of North Carolina	100.00
N. C. Section, American Chemical Society	75.00
Carolina Power and Light Company	175.00
Southern School Supply Company, Raleigh	156.50
Gifts of Academy members	6.50
Cardinal Products, Durham	150.00
J. D. Taylor for Denoyer-Goeppert Company	50.00
Carolina Biological Supply Company	50.00
Entry fee (in advance) for 1958 State Science Fair	25.00
Total Receipts for Science Fair Program	2128.00
Total Receipts for Academy and Science Fairs	4288.58

Expenditures—Academy proper	
Derieux Memorial Award—to Collegiate Academy	50.00
Research Grant (through A.A.A.S.)	25.00
Cancas (Collegiate Journal), two issues	113.25
Postage, Stationery, Office Supplies	237.41
Expenses of Annual meeting, 1957	20.30
Printing	138.00
Bank charges	0.18
Secretarial aid	85.90
Secretary's commission	100.00
Secretary's travel expense	42.60
Telephone (long distance calls)	42.30
Reprints of 1956 Proceedings	7.35
Expenses for representation at A.A.A.S. in New York	50.00
Expenses for representation at Junior Academy Conference in Chicago (repaid by A.A.A.S.)	125.00
Fidelity Bond (1 yr.) for Treasurer	5.00
Elisha Mitchell Scientific Society	557.50
Total for Academy proper	1599.79
Expenditures—Science Fair Program	
Slides from Science Clubs of America	42.00
Medals from Science Service	21.50
Ten copies of *High School Journal*, Chapel Hill	4.00
Announcement brochures, 1957 Fairs	106.50
Pictures made at 1957 State Fair	8.45
Entrance Fee, National Science Fair	175.00
To N. C. State College, for State Fair Expense	100.00
Plane Fare for National Fair party	1031.16
Personal expense for party, including transportation of exhibits	273.75
Prizes at State Science Fair	200.00
Prizes at North Central District Fair	100.00
Luncheon for planning of 1958 Fairs	26.32
Travel for persons at above luncheon	56.70
Total for Science Fair Program	2145.38
Total Expense, Academy and Science Fair Program	3745.17
Bank Balance as of July 1, 1957 578.41	
Less outstanding checks 35.00	
Adjusted bank balance	543.41
Total to account for	4288.58
Savings Account (Security National Bank, Raleigh) Balance, July 1, 1956	8.78
No interest to July 1, 1957	none
Present balance	8.78

Total Assets

Checking account (adjusted)	543.41
Savings Account	8.78
Raleigh Building and Loan Account	328.02
Total	880.21

On the 24th of August, 1957, we, the undersigned, examined this account and found it to be correct.

(Signed) E. W. GLAZENER, *Chairman*
WM. P. INGRAM, JR.
ERNEST O. BEAL

THE POTEAT AWARD

The award for this year was given to the best paper presented before the Biochemistry-Physiology Section. The award was won by Dr. Knut Schmidt-Nielsen of Duke University for his paper, "Extrarenal excretion of salt in birds". The certificate will be presented at the Academy banquet next year, 1958.

REPORT OF THE CONSERVATION-LEGISLATIVE COMMITTEE

The Conservation-Legislative Committee held only one meeting during the past year, and has not drafted any specific recommendations or resolutions to present in this session of the Academy. However, the Committee welcomes any suggestions or comments from the Academy relative to items that might, or should, be included on agenda of future meetings.

Continuous contact with national legislation affecting Conservation has been maintained individually through various reports, such as the Conservation Report of the National Wildlife Federation. In the first two weeks of the 85th Congress, several hundred bills were introduced in both Houses of Congress affecting aspects of natural resource conservation in one way or another. Any individual reaction registered for or against specific legislation did not implicate the Academy. A few items of legislation of local or state interest have been observed, including the Cape Fear Basin development proposals and the Venus Fly-Trap legislation with which this Academy has been implicated in past years.

Dr. Lowry of this Committee has suggested the desirability of lending support to Nature Conservancy which has as its mission the saving of biological communities of all kinds as "outdoor museums", where animals and plants can be studied and enjoyed by us, by our progeny, and by the generations that follow. The Nature Conservancy is the only national membership organization primarily dedicated to a program of land acquisition for the above purpose. This Academy may wish to consider lending a hand in the preservation of areas representing some of the last remaining examples of the wild nature that once made up America. This program is eminently worthwhile. More than that, the custodianship and husbandry of these areas is our trust, for, in the words of Ruskin, "they belong as much to those who come after us, as to us, and we have no right by anything we do, or neglect, to involve them in any unnecessary penalties or to deprive them of benefits that were in our power to bequeath."

WANDA S. HUNTER
E. M. LOWRY
A. E. RADFORD
T. E. MAKI, *Chairman*

REPORT ON THE ACADEMY CONFERENCE

Delegates from the N. C. Academy to the Academy Conference were John A. Yarbrough and J. F. Dashiell. The Conference held its 1956 meeting on December 29 at the Hotel Sheraton-McAlpin, New York City, in connection with the A.A.A.S. meeting in the same city. Twenty-seven of the forty-one State and City Academies of Science were represented. The morning session was concerned with organizational business and reports of activities from the Academies represented. Two particular items deserve emphasis. The Junior Scientists Assembly is sponsored by the Academy Conference in the city where the A.A.A.S. meeting is held. Its purpose is to inform and inspire young scientists in junior and senior high schools. Speakers from the adult science group and exhibits by the students are important items on the program. The 1956 Assembly was held at the American Museum of Natural History in New York City. A fine response was noted by the leaders. Another item concerns an effort by both the Academy Conference and the A.A.A.S. to assemble material for a history of science in the United States. Each Academy is being asked to begin work on the development of a history in its own state. Dr. Clinton L. Baker of Tennessee is chairman of the Academy Conference Committee in this work. (Note a resolution reported from our own Executive Committee at this Wake Forest meeting relating to the history project.)

An afternoon session of the Conference heard a panel discuss problems of science education in the high schools and colleges. Speakers were Dr. I. E. Wallen of the Science Teaching Improvement Program, A.A.A.S., Dr. L. J. Thomas of the University of Illinois and Dr. Campbell of the National Science Foundation.

At the evening session and banquet a presidential address was given by the retiring president, Father P. H. Yancey, Spring Hill College, Mobile, Alabama, and officers were elected for the coming year. These were Mrs. Thelma Heatwole, Staunton, Virginia, president, Dr. John A. Yarbrough, Raleigh, N. C., president-elect, and Dr. John G. Arnold, Jr., New Orleans, Louisiana, secretary.

REPORT ON COUNCIL MEETINGS OF THE A.A.A.S.

Two meetings of the Council were held in the Hotel Statler, New York City, on December 27 and 30. New officers who had previously been elected by mail ballot were announced as Wallace R. Brode, president-elect, Alan T. Waterman and Paul E. Klopsteg, members of the Board of Directors, and Karl M. Wilbur of Duke University as a member of the Nominating Committee. The dedication and use of the new A.A.A.S. headquarters building in Washington were significant events of 1956 for the organization. An unofficial poll of the Council on the matter of having the A.A.A.S. guarantee to each Council member a sum for travel expense to the annual meeting showed a majority of the affiliates opposed.

A significant item on the Council agenda was the report of the Committee on Social Aspects of Science. Considerable preliminary work has been done. There was much discussion in the Council on the matter of public dissemination of the views of scientists on matters that pertain to the social well-being of the nation.

It is obvious that all should carefully study these matters and their many implications. Dr. John A. Yarbrough represented the N. C. Academy at these sessions.

REPORT OF THE CHICAGO CONFERENCE ON JUNIOR ACADEMIES OF SCIENCE

The Secretary-Treasurer, Dr. John A. Yarbrough, was a delegate to this conference sponsored jointly by the Academy Conference, the Oak Ridge Institute of Nuclear Studies, and the A.A.A.S., with the National Science Foundation furnishing travel expense for the official delegates. The meeting was held on February 15 and 16 at Navy Pier, Chicago, with the Chicago branch of the University of Illinois acting as the host institution. Two general sessions were held, the first on Friday morning and the second on Saturday morning. Special area work conferences were held on Friday afternoon and these reported their findings and recommendations to the general session on Saturday.

A Junior Academy is an organization of students at the high school level (may include even fourth, fifth and sixth grades) to promote interest in science activities. Science fairs or exhibits may be a part of such a program but are usually supplemented by "paper sessions" in which students explain and describe their projects. The Junior Academy may operate independently of science fairs. In a variety of actual forms Junior Academies now operate in a number of states, e.g. Illinois, Alabama, Texas, Georgia, etc. Our own Executive Committee and newly organized High School Teaching Section are studying the Junior Academy idea for North Carolina. There is a possibility that it might supplement, but in no way supplant, our science fair program. In any case such an organization would be initiated and its operation carefully guided by the N. C. Academy of senior scientists.

A list of eleven concrete proposals grew out of the Chicago conference. These call for increased attention to the Junior Academy idea from State Academies and for help at the national level from A.A.A.S., the National Science Foundation and various science teacher organizations. Further promotion of Junior Academies is left largely to the Academy Conference.

REPORT ON THE COLLEGIATE ACADEMY

The activities of the Collegiate Academy for 1956–57 included:

1. A field trip on October 20, 1956. This consisted of a guided tour through Carolina Biological Supply Co. at Elon College, a brief business meeting, and a luncheon at Huey's Fish House. About thirty people were present, and the following colleges were represented: Atlantic Christian, Belmont Abbey, Duke, Elon, Meredith, Pfeiffer, and Woman's College, U. N. C.

2. A lectureship program for college science clubs. Dr. T. W. Johnson, Jr., of Duke University, was appointed faculty advisor for this project. Early in the fall he sent out a list of speakers in the fields of Botany, Chemistry, Geology, Mathematics, Physics, and Zoology. The object was to help to bridge the gap between the research scientist and the undergraduate. Thirty-five lectures were given under this program between October 15, 1956, and May 1, 1957. We hope

to continue this program next year, and wish to thank the administrative officers of Duke University and of the Greater University of North Carolina for providing travel funds for the speakers.

3. Two issues of *Cancas* were published, one in December and one in April. They contained abstracts of the papers given at the Collegiate Academy meeting last March, details of the lectureship program, reports of the various meetings, news of the clubs, etc. We are grateful to the Sigma Xi Chapters for their continued support.

4. The annual meeting held in connection with the N. C. Academy of Science. A social hour and luncheon this noon helped students from the various colleges to become better acquainted. At the meeting this afternoon a program of nine papers was followed by a business meeting at which a constitution was adopted and officers elected. A single award of $50, the John Bewley Derieux award, was given for the best paper, instead of two awards as heretofore.

5. The total paid-up membership for the year is 35 individual and 5 club memberships. The colleges represented this year are: Barber-Scotia, Belmont Abbey, Duke, Elon, Greensboro, Guilford, Meredith, Wake Forest, Western Carolina, and Woman's College, U. N. C.

CHARLOTTE DAWLEY

REPORT OF THE RESOLUTIONS COMMITTEE

Be it resolved that the North Carolina Academy of Science express its appreciation to President Tribble and his administrative assistants, to the faculty and to the students of Wake Forest College for their cordial hospitality at this, the fifty-fourth annual meeting of the Academy. To the chairman and members of the local committee and to the numerous persons who have cooperated with them we are grateful for the foresight and diligent efforts which have made this meeting proceed smoothly and comfortably in most pleasant surroundings.

Be it further resolved that a copy of this resolution be incorporated into the minutes of the Academy for 1957 and that copies be sent to President Tribble and to the Chairman of the Committee on Arrangements at Wake Forest College.

MAURICE WHITTINGHILL

The above resolution was unanimously approved by the Academy.

Memorials to the following members were read before the Academy: Eugene Willis Gudger, Arthur Sperry Pearse, and Samuel Thomas Emory. The memorials were approved by a rising vote of the members.

EUGENE WILLIS GUDGER

Eugene Willis Gudger was born at Waynesville, North Carolina, August 10, 1866. He died February 19, 1956, just short of ninety years of age. For fifty-one of those ninety years he was a member of the North Carolina Academy of Science. It is a tribute to his mind and character and zeal for scholarship that he became a highly educated man who devoted his life to the promotion of

learning although his boyhood was spent in troubled times just after the Civil War.

In spite of the paucity of educational opportunity in his youth he managed to get college preparatory education and entered Nashville College, and was awarded the B.S. degree in 1892 and the M.S. degree in 1893. After earning some money by teaching high school in Arkansas he entered the Johns Hopkins University as a graduate student in zoology. After three years of diligent work he received the degree of Doctor of Philosophy in 1905 at the age of thirty-nine. The autumn after obtaining his doctorate he became Professor of Biology at the North Carolina College for Women in Greensboro, a post he held for fifteen years. The remainder of his active scientific life was associated with the American Museum of Natural History where he was bibliographer for fishes, and Associate Curator.

Shortly after going to Greensboro, Dr. Gudger became a member of the North Carolina Academy of Science. He served as Secretary for eleven years. Because of his scholarly attainments, his splendid service as Secretary, and the esteem in which he was held, he was elected President in 1918. He retained his membership and his interest in the Academy after moving to New York.

As a scientist, Dr. Gudger was an independent and indefatigable worker. He went his own way doing what he thought should be done by someone, and he helped in the advancement of biological science. He investigated and recorded facts related to the morphology, embryology, habits, life histories and distribution of fishes. He also dug out of the literature and brought to more general attention all sorts of more or less lost observations of others, facts that sometimes were important, sometimes merely odd and interesting. His most lasting major contribution to the data of science probably was the compiling of the monumental bibliography of fishes.

Dr. Gudger's diligence and intelligent devotion to science won the respect and esteem of his colleagues and his sincere personality and friendly manner won the friendship of his associates. In his death the North Carolina Academy of Science mourns the passing of one of its earliest and greatly esteemed members.

ROBERT E. COKER
W. CRITZ GEORGE

ARTHUR SPERRY PEARSE

Arthur Sperry Pearse, Emeritus Professor of Zoology at Duke University, died December 11, 1956, in Durham, North Carolina, at the age of 79 years. He was born March 15, 1877, near Crete, and his boyhood was spent in various places in Nebraska and in Denver, Colorado, where he experienced the difficult life of western mining camps and pioneer farms of the period. From the very beginning he was intensely interested in natural history and outdoor life, and it is not surprising that he became an ecologist. He loved animals and, as he himself said, he was born to be a zoologist. He began collecting in his early years and had published several scientific papers before he completed his undergraduate work.

He interrupted his studies at the University of Nebraska to serve with the 4th Nebraska Volunteers in the Spanish-American War, returning to the University to receive the bachelor of science degree in 1900. He later earned his master's degree there under H. B. Ward, who gave him his fundamental training in parasitology. In 1941 the University of Nebraska honored him with an LL.D. degree.

After receiving his Ph.D. degree at Harvard University under the guidance of the late George H. Parker, Pearse began an active career in parasitology and ecology which carried him from the shores of Lake Michigan and Lake Mendota in Wisconsin to the Gulf of Mexico, to the Philippines, to the rain forests of Colombia and Venezuela in South America, to the cenotes and caves of Yucatan, to the Dry Tortugas and Bahamas, on to Japan, China, Siam and India, and to England and Nigeria. In 1930 he was Visiting Professor at Keio University in Japan.

Pearse's early affiliation, for three seasons, with the invertebrate zoology course at the Woods Hole Marine Biological Laboratory acquainted him with marine life and furnished the background for much of his later research on the ecology of marine fauna, a field in which he made perhaps his greatest contribution.

Twice he served with the International Health Board of the Rockefeller Foundation, first as a special member of a research team investigating hookworm infection in Alabama, and later, after a period of study at the London School of Tropical Medicine, as ecologist with a group working in Nigeria on the transmission of yellow fever.

Before joining the staff of the new Duke University in 1926 as Graduate Professor of Zoology, Pearse had held teaching positions at the University of Michigan, the University of the Philippines, St. Louis University School of Medicine, and the University of Wisconsin. At Michigan he served as Director of the biological station at Douglas Lake in the summer months. At Wisconsin, he contributed extensively to the biology of freshwater fauna.

While teaching in the Zoology Department of Duke University, his interest in marine biology led to the founding of the Duke University Marine Laboratory at Beaufort, N. C., in 1938. The station, in its present state of development, has largely followed the plans he originally envisioned. Pearse served as director of the station for ten years.

Pearse's broad range of interests is perhaps best seen by a glance at his list of publications, numbering about 175. These touch on such varied fields of research interest as parasitic crustaceans, freshwater fishes, animals of sea beaches and estuaries, the relationships between animals living in association with one another, and the fauna of soil and forest environments. An authority on Crustacea, he published numerous papers on parasitic copepods of marine fishes. An underlying theme of much of his work in marine ecology was the migration of animals from sea to land. Interest in this was aroused while studying the crustaceans of the Santa Marta region of Colombia, and henceforth this problem was ever before him as he traveled in other parts of the world. His book, *Emi-*

grations of Animals from the Sea, summarizes much of this research and shows his originality of thought. Among other books written by him is his *Animal Ecology*, a pioneering textbook in its field at the time, which demonstrated his depth of knowledge of animal associations. Pearse was instrumental in founding *Ecological Monographs* and was its editor for twenty years.

Pearse was a member of many scientific societies and served as president of several, including the Ecological Society of America, American Society of Zoologists, Association of Southeastern Biologists, and the North Carolina Academy of Science, and was vice president of the American Association for the Advancement of Science and chairman of Section F.

As a teacher, Pearse was unorthodox in many ways, yet his students, both graduate and undergraduate, were devoted to him. Heavily endowed with the adventurous spirit of the pioneer, he instilled in his students his own love of field work and his total disregard of the discomforts often attendant on it. His generosity to students, and, indeed, to anyone in need, was well known.

An outstanding scientist, an ardent research worker, and a stimulating teacher, he contributed much to the advancement of the field of ecology, not only in his writings, but also in the inspiration he gave to others. His aim was always to promote more effective teaching, research and scholarship.

I. E. GRAY

SAMUEL THOMAS EMORY

With the death of Samuel Thomas Emory on March 6, 1957, the State and the University of North Carolina lost one of their most valued and irreplaceable fellow citizens.

Sam Emory was born October 3, 1896, at Chase City, Virginia, but he lived most of his later years in North Carolina. After graduating from Randolph Macon College in 1917 with the degree of Bachelor of Arts, he continued his education first at Randolph Macon and then at Columbia University, receiving a Master of Arts degree from each of these institutions. His formal education was completed at the University of Chicago where he received his Doctorate in 1939.

In his early days he was much interested in the lower and intermediate levels of education, and served the people of the State long and well, first as Principal of the Goldsboro High School, and then as Superintendent of the public schools of Tarboro. He came to the University of North Carolina as Assistant Professor in 1933, was made full Professor twelve years later and, at the time of his death, had been serving as Chairman of the Department of Geology and Geography since 1949.

Sam Emory will be longest remembered for his extraordinary ability as a teacher and inspirer of youth. His deep interest in the welfare of his students is illustrated by his long services as adviser in the General College and as a member of its Administrative Board. He was a rare combination of old style "Southern Gentleman," clear-minded scientist, and well integrated citizen of the modern "Atomic Age" world.

His chief interest lay in the border zone between geography and history, so that he rapidly became an expert in Historical Geography, especially that of North America. His chief secondary interest was Political Geography. His somewhat limited bibliography in no way reflects his scholarly attainments. Sam was so much of a perfectionist that he hated to publish anything which had not been polished and perfected to his utmost ability. His genuine flair for administrative work, combined with his deep sense of duty to his community, led him to accept membership in almost innumerable committees of the University, the State, various national professional groups, his church, and the local community. This work, added to the duties of Department Head, left little time to devote to the leisurely re-working and polishing of manuscripts which he felt was so necessary. Had he lived, he was to have given up the chairmanship of his department at the end of the spring semester, and had planned to devote his time and energy to the completion of a book for which he had already accumulated a large file of data and chapter outlines.

There is no need to recite here the many honors which came his way. They are enumerated in the pages of such publications as "Who's Who" and similar directories of eminent persons. He was an active member of many professional and honor societies and often served on important committees. One of the last rewards he received, and one which he seemed to appreciate above all others, was his appointment in 1954 as Fulbright Lecturer at the University of Helsinki, Finland.

Samuel T. Emory will be long remembered with deep affection by his colleagues, his numerous personal friends, his former students, and the people of his community and his State as one of the finest, most scholarly, hardest working, and most worth-while friends of their entire lifetime. One of his former students wrote, "he represents the scholarship, friendliness, charm of manner and graciousness that were characteristic of Chapel Hill."

GERALD R. MACCARTHY
DAVID G. BASILE

SCIENCE EDUCATION IN N. C. HIGH SCHOOLS

The third year of the Academy's activity in this field with its new program was perhaps its most successful one to date. Seven district science fairs were held, followed by a State Fair at N. C. State College on April 25–27. Two winners at the latter fair were sent to Los Angeles with all expenses paid to compete in the National Science Fair. Both students gained national honors. William Montgomery of Myers Park High School, Charlotte, won a third place award in Physical Science and Betty Lou Wallace of Mountain Park High School won a fourth place award in Biological Science.

In the Third Annual North Carolina Science Talent Search two winners were recognized: Grayson H. Walker, Hayesville High School, Hayesville, who had already received an honorable mention in the national competition of the Westinghouse Talent Search, and Glendon Carlyle Smith, A. L. Brown High School, Kannapolis. Eleven other seniors from North Carolina high schools were listed

on the N. C. honorable mention group. For all students, recommendations were given to colleges of their choice and in some cases they obtained fine scholarship aid as a result.

The N. C. Talent Search Committee which selected the 1957 winners was composed of Dr. B. B. Brandt, chairman, Dr. J. M. Clarkson and Mr. Henry A. Shannon.

The Science Fairs for 1957 were conducted by Dr. Hollis J. Rogers for the third year as State Chairman. Chairmen of the District and State Fairs were as follows: Northeastern District, Dr. A. D. Bond, East Carolina College; North Central District, Dr. H. D. Crockford, U. N. C.; Southeastern District, Professor Louis Adcock, Wilmington College; North Piedmont District, Dr. Charles S. Black, Wake Forest College; South Piedmont District, Professor W. A. Parker, Catawba College; Northwestern District, Dr. Ray Derrick, Appalachian State Teacher's College; Western District, Professor J. G. Eller, Western Carolina College; N. C. State Science Fair, Dr. Ernest O. Beal, N. C. State College.

STATE SCIENCE FAIR WINNERS

Biological Science

(*Sponsored by Carolina Biological Supply Company and Mr. J. D. Taylor of Denoyer-Goeppert Company*)

First Prize: Betty Lou Wallace, Mountain Park High School, Mountain Park
Second Prize: John Sherrill, Acme-Delco High School, Delco
Third Prize: Susan Powell, Apex High School, Apex

Physical Science

(*Sponsored by Cardinal Products, Inc.*)

First Prize: William Montgomery, Myers Park High School, Charlotte
Second Prize: Johnny Hawkins, Enka High School, Candler
Third Prize: Cal Jowers, Boyden High School, Salisbury

Special Award in Geology

(*Sponsored by the Carolina Geological Society*)

Jannette Carringer, Andrews High School, Andrews

All participants in the State Science Fair were required to present from their district an entry fee. Sponsors in the seven districts who underwrote these entry fees and other sponsors of the Fairs included: Merck and Company, Inc.; Southern School Supply Company; Carolina Power and Light Company; E. I. du Pont de Nemours and Co., Kinston; Winston-Salem *Journal* and *Sentinel*; *Durham Herald-Sun* Papers; Raleigh *News and Observer-Times*; Medical Society of North Carolina; Rocky Mount *Evening and Sunday Telegram; Wilson Daily Times; Kinston Free Press; Daily Reflector*, Greenville; Mecklenburg County Medical Society; Charlotte Engineers Club; Waccamaw Bank and Trust Co., Whiteville; Riegel Paper Corporation; Mead Corporation; Champion Paper and Fiber Com-

pany; Olin Mathieson Chemical Corporation; Enka Corporation; Dayton Rubber Company; N. C. Department of Public Instruction; N. C. Section, American Chemical Society; North Carolina State College; All District Host Colleges.

REPORT OF THE NOMINATING COMMITTEE

The committee, consisting of E. C. Cocke, B. W. Wells and F. G. Hall, submitted the following nominations:

President: E. W. Berry, Duke University
Vice-President: Victor A. Greulach, University of North Carolina
Member of Executive Committee: Ben H. Hill, High Point College
Member of Research Grants Committee: Ernest Ball, North Carolina State College

The above nominees were unanimously elected by the Academy. This concluded the business and the meeting was adjourned.

More than eighty members of the Academy, guests and friends enjoyed the Annual Dinner of the Academy which was served in the beautiful Magnolia Room in Reynolds Hall on the Wake Forest campus at 6:30 p.m. Vice-President Dashiell presided over the banquet and a brief session which followed. President Harold W. Tribble and Mrs. Tribble were honored guests and President Tribble brought official greetings from the host institution. The 1956 Poteat Award, presented annually by Phipps and Bird, Inc., Richmond, Virginia, was formally presented by President Harkema to Dr. Karl M. Wilbur of Duke University for a paper given last year in the Zoology section by Dr. Wilbur and two colleagues, Dr. D. Gordon Sharp and Tadashi Tsujii. The title of their paper was "An electron microscope study of the growing surface of mollusc shell". For the first time the John Bewley Derieux Memorial Award was given to a paper chosen as the best presented in the Collegiate Academy. At the banquet this presentation was made by Secretary-Treasurer Yarbrough to Miss Betty Nock and Miss Katherine King, both of Meredith College, for a paper titled, "Basal metabolism studies of college freshmen".

Following the banquet the members and friends adjourned to the auditorium in the Science Building where President Reinard Harkema presented his interesting and informative Presidential Address on "Medical Zoology in Alaska". Vice-President Dashiell presented Dr. Harkema and presided over the session. Following the lecture all members and guests were entertained at a delightful social hour in the social room of the new Science Building as guests of Wake Forest College.

The following officers were elected for the respective sections:

Biochemistry and Physiology: Chairman, Knut Schmidt-Nielsen; Secretary, Claude L. Yarbro
Botany: Chairman, E. C. Cocke; Secretary, W. J. Koch
Geology: Chairman, G. R. MacCarthy; Secretary, Duncan Heron
Mathematics: Chairman, H. M. Nahikian; Secretary, Annye E. Welch
Physics: Chairman, Walter Gordy; Secretary, R. M. Helms

Zoology: Chairman, H. E. Lehman; Secretary, Charles M. Allen

High School Teaching: Chairman, R. T. Hatley; Secretary, Miss Dorothy Wilson

The following constitute the personnel of the standing committees:

Executive: E. W. Berry, Victor A. Greulach, John A. Yarbrough, L. A. Whitford, Maurice Whittinghill, and Ben H. Hill

Research Grants: Maurice Whittinghill, H. J. Oosting, T. H. Eaton, and Ernest Ball

Dr. E. W. Berry, President of the Academy for 1958, announced the appointment of the following committees to serve for the next year:

Auditing: J. M. Clarkson, E. W. Glazener, W. P. Ingram, Jr.

Nominating: Reinard Harkema, D. B. Anderson, J. W. Lasley

Policy Committee for H. S. Science Activities: H. D. Crockford, Henry A. Shannon, Arthur Roe, T. D. Reynolds, Hollis J. Rogers, R. H. Loeppert, John A. Yarbrough

Conservation-Legislation: T. E. Maki, A. E. Radford, Wanda Hunter, E. M. Lowry

Resolutions: Ben H. Hill

North Carolina Science Talent Search: B. B. Brandt, J. M. Clarkson, Henry A. Shannon

Representative on A.A.A.S. Council: John A. Yarbrough

Representative on Academy Conference: John A. Yarbrough

The following papers were presented during the meetings at Wake Forest College. Those marked with an x are abstracted in these proceedings:

GENERAL SESSION

Recent advances in organic chemistry. Arthur Roe, Chairman of the Department of Chemistry, U. N. C.

Recent advances in geology. J. M. Parker, III, Department of Geology, N. C. State College

Ecology, today and yesterday. H. J. Oosting, Chairman of the Department of Botany, Duke University

COLLEGIATE ACADEMY

Some synthetic organic procedures directed toward the preparation of fluoroskatoles. Roy J. Duckett and Richard B. Simpson, Elon College

The analysis of a high-silica volcanic rock for the determination of the effect of weathering upon its chemical composition. Vincent Vitale, Jr., Elon College

Differentiation in roots of Hedera helix *L.* Ann Marie Stephenson and Heinrich Tschinkel, Duke University

Vascular plants of Wilson County, North Carolina. James F. Matthews, Atlantic Christian College

Hormones in the invertebrates. Melinda Speas, Wake Forest College

Basal metabolism studies of college freshmen. Katherine King and Betty Nock, Meredith College

Organolithium compounds—a review. Ikie N. Presson, Western Carolina College

Some organic preparations leading to the synthesis of 2,4-difluorophenylacetic acid. Patricia Kittinger, Elon College

A quantitative nephlometric technique for the evaluation of the precipitin reaction. Donald J. Wright, Duke University

BIOCHEMISTRY AND PHYSIOLOGY SECTION

x *The localization of basic proteins in the salivary chromosomes of* Drosophila. Edward C. Horn and Calvin L. Ward, Duke University

x *Localization in the amygdala.* Charles D. Wood, U. N. C.

x *The active site of xanthine oxidase.* I. Fridovich and Philip Handler, Duke University

The control of the phosphorylative rate in washed rat liver mitochondria. Marjorie A. Swanson, Bowman Gray School of Medicine

x *Approximation of "total vascular space" and its distribution in three sizes of blood vessels in rats by plastic casts.* William H. Knisely and Harriman H. Jett, Duke University

The counter-current principle and urea excretion. Bodil Schmidt-Nielsen, Duke University

x *Extrarenal excretion of salt in birds.* K. Schmidt-Nielsen, Duke University

Renal responses to diamox at several intervals after adrenalectomy and the effect of D. C. G. D. A. Olewine and J. H. Perlmutt, U. N. C.

x *Anti-AcG: a specific clotting-factor inhibitor.* John H. Ferguson and C. L. Johnson, Jr., U. N. C.

x *Studies on the structure of plasmalogens.* Cosmo A. DiFazio (introduced by Carl E. Anderson), U. N. C.

x *A further study of the action of diethanolamine on liver lipids.* Camillo Artom, H. B. Lofland and John Oates, Jr., Bowman Gray School of Medicine

A trimethylamine yielding compound produced from choline in rat liver preparations. H. B. Lofland, H. M. Salem and C. Artom, Bowman Gray School of Medicine

BOTANY SECTION

x *Progress in the development of root knot resistant varieties of sericea* Lespedeza (L. cuneata). T. E. Smith, McNair's Yield-Tested Seed Co.

Inheritance of seed size in watermelon, and the effect of seed size of the diploid and tetraploid parents on the size of undeveloped ovules (in seedless watermelons). P. R. Konsler and W. S. Barham, N. C. State College

x *Interactions of gibberellic acid and maleic hydrazide in relation to the growth of bean plants.* John G. Haesloop, U. N. C.

x *Reaction of excised corn root tips to gibberellic acid, kinetin, and maleic hydrazide.* Robert S. Leisner, U. N. C.

x *Further studies on the* Striga *parasite of corn.* R. R. Nelson and D. E. Ellis,N. C. State College

x *Further studies in the genus* Chytridium. William J. Koch, U. N. C.

Morphological variation in Nuphar *Sm.* Ernest Beal, N. C. State College

Genotypic reactions to boll-weevil attack in Upland cotton. Wolfgang H. Wessling, N. C. State College

Further evidence on the importance of the current effect. L. A. Whitford, N. C. State College

Venus' flytrap and its environment. Patricia R. Roberts, Duke University

GEOLOGY SECTION

x *The occurrence of salty ground water in the Coastal Plain of North Carolina.* H. E. LeGrand and P. M. Brown, U. S. Geological Survey, Raleigh

x *Size properties of the sediments of Newport River Bay, Morehead City, N. C.* Fritz K. Johnson and Roy L. Ingram, U. N. C.

x *Clay minerals of some Carolina bay sediments.* Roy L. Ingram and Maryanne Robinson, U. N. C. and Duke University

MATHEMATICS SECTION

Computation of the greatest positive characteristic root of a positive matrix. Alfred Brauer, U. N. C.

X's and Θ's applied to building frames. T. F. Hickerson, U. N. C.

x *Mathematical postage stamps.* Ralph B. Johnson, Catawba College

Curvature and torsion. John W. Sawyer, Wake Forest College

PHYSICS SECTION

Detection of heavy primary cosmic rays by means of large silver chloride crystals. Charles B. Childs, U. N. C.

Effect of dislocations on the optical properties of silver chloride crystals. John Harden, Jr. U. N. C.

Effects of ultraviolet irradiation upon certain mechanical properties of AgCl single crystals. Milton N. Kabler, U. N. C.

Atomic mobility in solids; concentration gradient anomalies. William G. Mallard, U. N. C.

Effect of atomic size on atom mobility in metal crystals by radioactive tracer technique. George P. Williams, Jr., U. N. C.

x *Tabulation of the Wigner 9j-coefficients.* J. W. Stephenson and K. Smith, U. N. C.

x *A study of perturbation methods in nuclear reactors.* E. Rudolph Prince, Jr., N. C. State College

x *Computer solutions for nuclear reactor kinetics problems.* Albert T. McMain, Jr., N. C. State College

A state committee on Mathematics and Science Education. Walter M. Nielsen, Duke University (invited speaker)

Superconductivity. Rolfe Glover, U. N. C. (invited speaker)

ZOOLOGY SECTION

Carrot rust fly control with herbicidal oil plus insecticides. H. Eldon Scott, N. C. State College

x *Carbon dioxide fixation by the oyster.* Carl S. Hammen, Duke University

The distribution of the fishes of the Saluda River drainage basin, South Carolina. F. Ray Derrick, Appalachian State Teachers College

x *The diagenetic efficiency of some Arctic lakes.* D. A. Livingston, Duke University

Pre-Linnean binomial system and other features of Carolina Indian ethnology. A. L. Pickens, Presbyterian Junior College

x *Some measurements of diurnal oxygen metabolism in streams.* Charles M. Hoskin, Duke University

Variation in the snake Rachidelus brazili. Joseph R. Bailey, Duke University

x *Use of antibiotics for obtaining bacteria-free cultures of* Arcella. J. E. Davis, Wake Forest College

The effect of chelation on X-ray induced ovarian damage in the wasp Habrobracon. Leo Emery LaChance, N. C. State College

x In vitro *studies of active sodium and potassium transport in mouse ascites tumor cells.* Edgar F. Stillwell, Duke University

x *The cytotoxicity of an antiserum prepared against the supernatant fraction of the Ehrlich ascites tumor cell.* Edward C. Horn and Mary Elizabeth House, Duke University

x *Effects of depletion and substitution of perivesicular mesenchyme on the development of the thyroid primordium.* William E. Dossel, U. N. C.

x *An immuno-histological investigation of tissue antigens in the frog embryo.* William M. Clarke, Duke University

x *Maternal antigens in mature frog oocytes as demonstrated by fluorescent antibody.* Laurel E. Glass, Duke University

x *A cytological analysis of salamander hybrids from the cross,* Diemictylus (Triturus) viridescens x Taricha (Triturus) granulosa. L. M. Youngs and H. E. Lehman, U. N. C.

x *A preliminary report on extrinsic factors influencing pigment cell morphology.* H. E. Lehman and L. M. Youngs, U. N. C.

HIGH SCHOOL TEACHING SECTION

The purpose and possibilities of the summer science institutes. Thomas D. Reynolds, Duke University

My experiences in two science institutes. Mrs. Mary J. Fisher, Concord High School

Possibilities for a Junior Academy of Science in North Carolina. John A. Yarbrough, Meredith College

Available war surplus material for science departments. R. C. Hatley, Albemarle High School

The Localization of Basic Proteins in the Salivary Gland Chromosomes of Drosophila.[1] EDWARD C. HORN AND CALVIN L. WARD. Salivary gland preparations from both sexes of *Drosophila virilis* larvae were stained with a modified Feulgen

[1] This work was supported in part by research grant RG-4203 from the National Institutes of Health, Public Health Service.

reaction and selected chromosomes were photographed and located with a calibrated stage. These same preparations were then re-exposed, extracted with hot TCA and stained according to the method of Alfert and Geschwind with fast green at controlled pH to identify the basic proteins. Photographs were then taken of the identical chromosomes recorded after Feulgen stain and band-by-band comparisons made.

At least five relevant observations have been made: 1. The localization of deoxyribose nucleic acid appears to be identical to that of the basic protein in the banded regions, the stains for the two coinciding and leaving interband spaces colorless. 2. The Feulgen-negative nucleolus is faintly positive with fast green, indicating the presence of some basic protein. The Feulgen-positive nucleolar organizing region is strongly positive with fast green. 3. Regions of diffuse heterochromatin give not only a positive Feulgen but also a positive basic protein reaction. 4. The cytoplasm of the glands is also positive to the basic protein test, indicating either the unexplained presence of basic protein in the cytoplasm or, more likely, another substance positive for this test. 5. Unbroken nuclei treated only with the fast green technique are outlined with positively staining material which may or may not be the nuclear membrane. In these same preparations the interchromosomal space lacks any detectable histone.

Localization in the Amygdala. CHARLES D. WOOD. Emotional and behavioral changes have been produced by stimulation or destruction of the amygdaloid complex. The amygdaloid complex is composed of nuclei of diverse embryology, phylogeny, and histology; however, localization of function within the complex is questioned.

Stimulation of the amygdaloid region in conscious or lightly anesthetized cats and rats gave variable respiratory and blood pressure responses. With surgical levels of anesthesia, respiratory inhibition and a fall in blood pressure were consistently elicited. These responses were much more pronounced and were produced with lower stimulation voltages in the medial division of the amygdala than from the lateral division. Lesions of the ventromedial hypothalamic nucleus abolished significant blood pressure responses and raised the threshold of the respiratory responses. The tracts mediating the blood pressure responses appear to be associated with the ventromedial hypothalamic nucleus to a greater extent than those mediating the respiratory responses.

Lesions of the lateral amygdaloid nucleus in cats produced a permanent hypersexed state. It has been reported that stimulation of the medial amygdaloid areas caused ovulation and uterine contractions. This suggests that the medial and lateral divisions of this region may have opposing influences. Lesions of the central nucleus produced aggressive behavior while stimulation of the basal and central nuclei evoked anger or fear. These behavioral changes may be a result of altering the balance of the influence between the divisions of the amygdala.

The Active Site of Xanthine Oxidase. IRWIN FRIDOVICH AND PHILIP HANDLER. Xanthine oxidase exhibits two distinct modes of reaction with *p*-chloromercuribenzoate (PCMB), one being rapid and virtually complete in a few minutes while

the second is slow and continues for several hours. It may be considered that the rapid reaction involves free sulfhydryl groups while the slow reaction involves chemically hindered sulfhydryl. In view of the iron content of xanthine oxidase it appeared possible that the hindered sulfhydryl groups were relatively unreactive by virtue of being bonded to a metal, possibly iron, and that the slow reaction involved a competition between the mercurial inhibitor and the enzyme bound metal for the sulfur bond. If the metal of xanthine oxidase is involved in its enzymatic activity then the hindered sulfhydryl should represent an active site while the freely reacting sulfhydryl need not. Furthermore, the interaction of the proposed site with substrate should result in reduction of the sulfur metal bond and consequent liberation of a freely reacting sulfhydryl. The inhibition of xanthine oxidase by PCMB and its reversal by cysteine has been investigated under varying conditions in the presence and absence of substrate and the results confirm the above hypotheses. The inhibition of xanthine oxidase by cyanide, which has long been recognized as being peculiar in several respects, may also be explained in terms of a sulfur-metal active site and here, too, experimental results tend to confirm the proposed structure of the active site.

Approximation of "Total Vascular Space" and its Distribution in Three Sizes of Blood Vessels in Rats by Plastic Casts.[2] WILLIAM H. KNISELY[3], M. S. MAHALEY, JR., AND HARRIMAN H. JETT. There are many conditions in which it would be useful to know the "total potential vascular space" of animals or patients, and, how the total is distributed among various-sized blood vessels. Both vascular space and percentage in various vessels have been determined by serial sections for two dog organs; F. P. Mall (1888) measured both for small intestine and W. S. Miller (1893) for the lung. H. D. Green (1944) used Mall's data to *calculate* both for a "whole" animal.

Using liquid methyl methacrylate mixed with catalyst and promoter, vascular casts were made of four rats weighing 112–360 grams. The plastic was injected at less than 75 mm. Hg pressure via cannulated aorta until it ran out of the cut left ventricle. The polymerization shrinkage and specific density were determined, and from the weights, the volumes which the plastic had before polymerization were calculated. The casts were separated into "compartments" with a diameter of 150 μ and smaller, 150–160 μ, and 600 μ and larger, with forceps and a dissecting microscope with a calibrated ocular scale.

Gross and microscopic inspection and comparison with *in vivo* observations of a number of organs showed all organs equally filled, some capillaries not filled in all parts, and that the veins were not maximally distended. The "total vascular spaces" found were from 8.9–11.1 cc. per 100 grams body weight. The smallest is larger than the largest blood volume determined for the rat in the literature, 7.98 cc. per 100 grams (Beckwith and Chanutin, 1941). The means of the percentages in the 3 "compartments" were 10.4 in vessels 600 μ and larger, 6.7 in those 600–150 μ, and 82.9 in vessels 150 μ and smaller.

[2] Supported by grants from the Duke University Council on Research and the Durham-Orange County Heart Association.

[3] Done during the tenure of an American Heart Association Research Fellowship.

The finding of 82.9 per cent in casts 150 μ and smaller is important because of the immensity of the figure and because of the known variation in hematocrit between large and small vessels.

In our opinion a more accurate approximation will be larger, because we know there were uninjected capillaries, and the veins were not filled to their maximum capacity.

Extrarenal Excretion of Salt in Birds. KNUT SCHMIDT-NIELSEN. It has often been suggested that marine birds must drink sea water in order to cover their normal needs for water. This problem was investigated, using young cormorants as experimental animals.

The investigations gave no evidence for the necessity of drinking sea water, because the water content of fresh fish is more than adequate for the elimination of salts and nitrogen (uric acid). However, it was found that the cormorants used in these investigations have an extra-renal mechanism for the elimination of salt.

During experimental salt loads, whether imposed orally or by infusion of hypertonic sodium chloride solution, cormorants react by excreting a highly hypertonic liquid from the nose. The secretion drips out from the internal nares and collects at the tip of the beak, from which the bird shakes the drops with a sudden jerk of the head.

The concentration (500–600 mN NaCl) and the rate of secretion (up to 0.2 ml. per min. in a 1.5 kg. bird) are so high that with continuous secretion the entire NaCl content of the body would be eliminated in roughly ten hours. The secretion contains practically only sodium and chloride in nearly equivalent amounts (potassium was found to be about 10 mN, magnesium 2–3 mM, and sulfate 2–3 mM).

The production of the nasal secretion is stimulated not only by NaCl, but also by a non-electrolytic osmotic load (sucrose). The secretion obtained in response to such stimulation is similar in composition to that obtained by stimulation with NaCl, indicating that the mechanism responds to general osmotic conditions, rather than specifically to the concentration of the sodium or chloride ions in the plasma. (Supported by Grant H-2228 from the National Institutes of Health.)

Anti-AcG: a Specific Clotting-Factor Inhibitor. JOHN H. FERGUSON AND C. L. JOHNSTON, JR. With recent knowledge of a number of protein factors normally participating in the mechanisms of blood clotting, it is understandable that specific inhibitors (? antibodies) acting against any of these proteins might develop. There is evidence (Blood, 1956), for instance, that anti-AHF or anti-PTC occasionally appear, following transfusions, in hemophiliacs or PTC-deficients, respectively. A recent case is that of an elderly white male who developed a bleeding tendency (hematuria) following a gall-bladder operation and seemed to be made worse by transfusions. The following tests established the presence of a circulating inhibitor specific for AcG (labile factor, factor V):

The prothrombin time (Quick test) was prolonged, and, initially, also in mixtures of patient's and normal plasma. A partial correction was afforded by (1) dilution, (2) AcG (bovine serum, adsorbed with barium carbonate), and (3)

latterly, in mixtures with the plasma of two normal daughters. Specific AcG assays were less than one per cent, and powerful inhibition, in these tests, was shown in mixtures of patient's plasma with (a) AcG or (b) fresh normal plasma. Using (a), at AcG strength comparable to that of (b), and testing dilutions of patient's plasma, an inhibitor titer was determined at about 1:160. The inhibitor was relatively stable, not adsorbed by barium sulfate, and quantitatively recovered in ammonium sulfate fractions between 25 and 50 per cent saturation. This indicates it to be a globulin protein.

The presence of the inhibitor results in: (1) prolonged whole blood and plasma recalcification clotting-times, (2) prolonged prothrombin time, (3) prolonged partial thromboplastin times, (4) abnormal thromboplastin generation tests, (5) deficient prothrombin consumption on some (not later) occasions. Suitable laboratory tests showed normal levels of platelets, fibrinogen, prothrombin, proconvertin, and correction of the deficiencies in hemophilic and PTC-deficient plasmas. Tests for anti-proconvertin, and for heparin (protamine titration) were negative. The only significant defect, therefore, was lack of AcG attributable to the presence in the circulation of a powerful inhibitor specific for this clotting factor.

Studies on the Structure of Plasmalogens. COSMO A. DI FAZIO, CARL E. ANDERSON AND E. A. BRECHT. Acetal phosphatides isolated after alkaline hydrolysis of tissue phospholipids may differ from the natural plasmalogens. Klenk, and later Rapport, have indicated that plasmalogens contain both a fatty acid and aldehydogenic moiety combined with the glycerol. Hydrogenation followed by saponification of tissue phosphatides led to the isolation of batyl alcohol phosphate. This finding led Klenk to postulate that a hemiacetal might be linked to the alpha position of glycerol. Baer proposed an anhydride structure between an acetal in the primary position and an ortho-ester in the beta position of glycerol, indicating that the cyclic structure could form following hydrolysis of the secondary ester with mild alkali.

In order to evaluate Baer's hypothesis, the conditions for cyclic acetal formation were investigated, utilizing the transacetalation procedure of Piantadosi. Kinetic studies of this reaction showed that the first step, the formation of the mixed glycerol acetal was acid catalyzed and involved a carbonium ion mechanism. The conversion of the mixed acetal to the cyclic glycerol acetal was dependent upon heat only, temperatures in excess of 100°C. being required for cyclization. These findings indicate that the formation of the cyclic structure during mild alkali treatment at lower temperatures (37°–80°C.) as proposed by Baer is unlikely.

Acid hydrolysis of cyclic acetals was next studied. The following cyclic acetal derivatives of palmitaldehyde were synthesized and their rates of hydrolysis in various acid media determined: the ethylene glycol acetal, 1,2-propanediol acetal, 1,2-glycerol acetal, 3-methoxy glycerol acetal, 3-chloropropanediol acetal, acetal phosphatidic acid and acetal phosphatide. The rate of hydrolysis of these cyclic acetals decreased as the electron withdrawing effect of the substituent groups increased. The acetal phosphatidic acid and acetal phosphatide hydro-

lyzed only upon treatment with a mineral acid. The hydrolysis of the open chain dimethyl acetal was complete within a matter of minutes when treated with mild acid (90 per cent acetic acid).

Since tissue plasmalogens readily yield an aldehyde when treated with mild acid, it is possible that tissue plasmalogens contain both the cyclic acetal structure and open chain structures such as those proposed by Klenk and others.

A Further Study of the Action of Diethanolamine on Liver Lipides. CAMILLO ARTOM, H. B. LOFLAND AND JOHN OATES, JR. In a reinvestigation and extension of previous results (Artom, Cornatzer and Crowder, 1949, Jour. Biol. Chem. **180:** 496), it was confirmed that diethanolamine added at the level of 0.5 per cent to various types of diets low in protein causes marked changes in the composition of rat liver lipides. These changes consist chiefly of an increase in the non-choline-containing phospholipides and a decrease, both absolute and relative, in the choline-containing fraction. Opposite effects are obtained when the low protein diets are supplemented with choline. However, in the liver of rats receiving either diethanolamine or choline, the amounts of neutral fat are lower than in the livers of the controls fed the unsupplemented diets.

Evidence for the incorporation of C^{14}-diethanolamine into liver phospholipides was obtained, both *in vivo* and *in vitro*. After injection in the intact animals, the radioactivity of the liver lipides reaches its peak between the third and the sixth hours and then declines slowly. Under the same conditions, a much larger proportion of C^{14}-ethanolamine is incorporated into the liver lipides. With slices or homogenates of the liver, C^{14}-diethanolamine is incorporated into the lipides to about the same extent as labeled ethanolamine or labeled choline. The lipides extracted from the liver of rats receiving C^{14}-diethanolamine were hydrolyzed and the components were separated by paper chromatography. The results indicated that most of the radioactivity was present in a position corresponding to that of pure diethanolamine. It seems likely that diethanolamine is readily incorporated into the phospholipides, but that these atypical cephalins are metabolized more slowly than their natural analogues and therefore accumulate in the liver, thus accounting for the increase in the non-choline-containing fraction.

A comparison between the lipide composition of rats receiving diethanolamine alone, or in combination with either choline or methionine, does not support the hypothesis that diethanolamine causes an impairment of the synthesis of choline, because of a decreased availability of ethanolamine as the methyl acceptor. The findings that the livers of rats receiving diethanolamine contain only moderate amounts of neutral fat and that homogenates of such livers oxidize very actively fatty acids added *in vitro* (Artom, 1956, Federation Proc. **15:** 213) also suggest that a severe deficiency of choline does not occur. Accordingly, it seems more probable that the decrease in the choline-containing fraction of the liver is due to a competitive inhibition of the synthesis of such phospholipides from choline. In line with this interpretation, it was observed that diethanolamine, added *in vitro* to liver slices or homogenates, strongly inhibits the incorporation of labeled choline into the lipides.

Progress in the Development of Root Knot Resistant Varieties of Sericea Lespedeza (L. cuneata). T. E. SMITH AND J. W. FLETCHER. Seed were harvested from 205 plants in old stands of sericea, Arlington and Common varieties, growing near Laurinburg in 1950. In 1951, a single plot was planted with each selection using seed from the apetalous, cleistogamous flowers because these are naturally self pollinated. Much variation occurred between the different progenies in root knot resistance and in plant type. Seed produced by the cleistogamous flowers were saved from individual plants of resistant selections for further work. Repeated tests since 1952 have isolated seven strains, descendants of three original single plant selections, with high resistance to common root knot (*Meloidogyne* sp.). Experiments to determine yield and forage value are in progress simultaneously with preliminary seed increases.

Interactions of Gibberellic Acid and Maleic Hydrazide in Relation to the Growth of Beans. JOHN G. HAESLOOP. Young bean plants, variety Black Valentine, were treated with 10^{-3} M. aqueous solutions of gibberellic acid and maleic hydrazide. One ml. of each solution was applied to the upper surface of the primary leaves of the experimental plants. Plants treated with gibberellic acid attained a height of as much as 2.5 times that of untreated plants. This was due to increased internode length and not to an increase in the number of nodes. The growth of plants treated with maleic hydrazide was completely stopped within a week after treatment and terminal bud abscission occurred on all of these plants. The height attained by plants treated with maleic hydrazide was no more than one half of that of untreated plants. Plants treated with both gibberellic acid and maleic hydrazide grew nearly as tall as untreated plants. This height was attained as a result of increased elongation of young internodes present at the time of treatment. The terminal buds abscissed within a week after treatment. The data obtained from these experiments indicate that gibberellic acid and maleic hydrazide are not antagonistic. Gibberellic acid causes an increase in growth by markedly stimulating internode elongation and maleic hydrazide stops growth by inhibiting cell division.

The Reaction of Excised Corn Root Tips to Gibberellic Acid, Kinetin, and Maleic Hydrazide. ROBERT S. LEISNER. Experiments were performed to determine whether either gibberellic acid or kinetin or both would overcome the inhibitory effects of maleic hydrazide. Structural formulae of the three compounds were obtained from the literature. Seeds of sweet corn, Golden Cross Bantam Hybrid Variety, were sterilized with 0.1 per cent mercuric chloride for 30 minutes, washed several times with sterile, distilled water, and soaked overnight in sterile, distilled water. Seeds were placed on sterile, moist filter paper in petri dishes to germinate. Each of the 125-ml. Erlenmeyer flasks contained as a basic medium, 50 ml. White's basic nutrient solution, 1.5 per cent agar, and 0.1 per cent solution containing the micro-essential elements B., Cu., Mn., and Zn. Test compounds were in aqueous solutions of 10^{-3} M. concentration and were used in the following concentrations: gibberellic acid, 7.0 and 14.0 μg./ml.; kinetin, 5.0 and 10.0 μg./ml.; and maleic hydrazide, 100.0 and 200.0 μg./ml. All

possible combinations of the three compounds were used. Preliminary tests were performed with gibberellic acid to determine whether autoclaving the compound would alter any of its properties. Similar results were obtained from both autoclaved and non-autoclaved gibberellic acid when used on bean plants. Root tips were excised 7 days after placing them on the filter paper, 5-mm. apical lengths being used. These tips were placed in the sterile flasks, 10 tips in each flask. The flasks were then placed in the dark at a temperature of 26°C. Measurements were taken every 24 hours for a period of 12 days. The criterion for growth was linear increase in the length of the root tips. Results of this experiment indicate that gibberellic acid has little or no effect on the elongation of the root tips, has a tendency to inhibit the formation of secondary roots, and does not overcome the inhibitory effects of maleic hydrazide. Kinetin inhibits the growth of root tips to a degree of about fifty per cent of that of maleic hydrazide; in combination with maleic hydrazide the inhibition is nearly complete and immediate; in combination with gibberellic acid inhibition is less than that with maleic hydrazide but greater than that of kinetin alone; and no secondary roots are formed on any culture containing kinetin. Maleic hydrazide is known to inhibit root tip growth but the concentrations used in this experiment were not high enough to give the classic inhibitory effects. Combinations of all three compounds gave results almost identical with those of the combination of maleic hydrazide and kinetin.

Further Studies on the Striga *Parasite of Corn.* R. R. NELSON AND D. E. ELLIS. Further investigations concerning *Striga* sp., a parasitic flowering plant attacking corn and other gramineous hosts in North Carolina and South Carolina, are presented. Initial surveys have located the parasitic weed in more than 80 infested areas in four counties in North Carolina and in 40 infested areas in the four adjoining counties in South Carolina. Present host range studies have demonstrated that 21 species of Gramineae representing 16 genera are hosts to *Striga*. Known hosts include such important cultivated crops as corn, brome grass, oats, barley, and millet as well as such commonly occurring grass weeds as crabgrass, goosegrass and Bermuda grass. No non-gramineous species have been shown to be hosts to the *Striga* species present in North Carolina and South Carolina. Studies suggest that species may vary in their degree of susceptibility. A small percentage of seed of *Striga* will germinate immediately upon harvest, although percentage germination is greatly enhanced with time. *Striga* can parasitize known hosts in a variety of soil types including sand, light clay, loam, sandy loam, and clay loam. Growth and development are more slow in heavier soils. Studies have shown that *Striga* can develop under a range of soil temperatures, although growth is most rapid at 30–35°C., which also is optimum for seed germination.

Further Studies in the Genus Chytridium. WILLIAM J. KOCH. A new species has been found on pollen and boiled leaf bait in soil from Alabama, Michigan, North Carolina, and South Carolina. It was thought to be a species of *Chytridium* because of features of its zoosporangial thallus; however, pure culture studies reveal that it forms resting spores by an unusual sexual method: Plasmogamy

occurs at the tips of rhizoids of two contributing thalli. A short tube grows out spirally from the point of fusion, and the tip of this tube swells or buds out. The nuclei of the two contributing thalli move out of the old spore cysts, and karyogamy occurs in the swelling. This initiates the formation of a new rhizoidal system. The swelling continues to enlarge and develops into a typical chytrid resting spore with an orange wall. In the known sexual species of *Chytridium* the resting spore forms in the apophysis of a receptive or female thallus after the protoplast of a male, contributing thallus enters through the old spore cyst of the female. Asexually formed resting spores develop in a similar way, without the aid of a male. This pattern of resting spore development is considered a basic feature of the genus *Chytridium*; thus, it is concluded that the new species must be considered the type of a new genus.

The Occurrence of Salty Ground Water in the Coastal Plain of North Carolina.[4] H. E. LEGRAND AND P. M. BROWN. The Coastal Plain of North Carolina is underlain by strata of sand, clay, marl, and limestone, which dip and thicken toward the coast, forming in aggregate a wedge-shaped mass. This wedge thickens from a featheredge at the inland margin of the region to several thousand feet near the Outer Banks. Sea water originally in the strata has been largely flushed out by fresh water recharged by precipitation on the elevated parts of the strata. The extent to which potable water has replaced salt water in the strata is dependent upon the following factors: geologic structures, the differences in density between salt and fresh water, the nature and location of recharge and discharge areas and the hydraulic continuity between them, and time. The uppermost strata, especially the more permeable ones, have been the most completely flushed of salt water, resulting in fresh-water strata lying above and in some places in contact with salt-water strata. If sea level is approximately stationary for a long enough time, the salt water will be replaced by fresh water to a depth that is about forty times greater than the height of the ground-water level above sea level.

Clay beds sufficiently impermeable to separate artesian water from unconfined, shallow ground water are generally found within fifty feet of the land surface in southeastern North Carolina. The surficial sands contain unconfined ground water that is fresh. However, in the surficial sands of the beaches and of land adjacent to the coastal waters, the water table may be so slightly above sea level that wells may reach salty water at comparatively shallow depths.

A map showing the approximate depth to artesian water containing more than 250 parts per million of chloride indicates the areal distribution of the body of artesian water that is fresh. Wherever water containing more than 250 parts per million of chloride is present at moderate depth, it is fairly safe to assume that no potable water will be found in strata at greater depth.

Withdrawal of ground water in North Carolina has not been sufficiently concentrated to cause encroachment of unpotable water from nearby salt-water bodies.

[4] Publication authorized by the Director, U. S. Geological Survey.

Size Properties of the Sediments of Newport River Bay, Morehead City, North Carolina. FRITZ K. JOHNSON AND ROY L. INGRAM. Data obtained from the study of 173 selected bottom samples indicate that the sediment distribution in the Newport River Bay is as follows: (1) upper third—clayey silt and silt in the quiet marginal waters and sand, sandy silt and scattered organic reefs in the central portion; (2) middle third—medium and fine sand along the margin and sandy silt and clayey silt in the central channel; (3) lower third—fine sand and scattered marshes consisting of oyster shells and trapped sandy silt; (4) transition zone between bay and ocean—medium and course sand.

The light sand fraction consist of quartz (93.6%), plagioclase (3.5%), and potash feldspar (2.8%). The heavy mineral content constitutes less than one per cent of each sample. The clay minerals consist of illite and "chlorite" with an increase in the "chlorite" seaward.

Clay Minerals of Some Carolina Bay Sediments. ROY L. INGRAM AND MARY-ANNE ROBINSON. The clay minerals from the sediments of White Lake, Singletary Lake, and three other small Carolina Bays near Laurinburg, N. C., were identified by X-ray diffraction methods. Variations from sample to sample and from lake to lake are insignificant. Kaolinite and a 14 A mica intermediate (?) or chlorite (?) predominate in most of the samples. A small amount of illite is present in some of the samples. All of these clay minerals are present in the surficial Pleistocene deposits of the North Carolina Coastal Plain, and the clay minerals in the Carolina Bays are interpreted as allogenic clays from these Pleistocene deposits.

Mathematical Postage Stamps. RALPH B. JOHNSON. The purpose of this paper, which consisted of a display and list, was to introduce the non-philatelist to those mathematical subjects which had been commemorated by postage stamps.

A display of one hundred and twenty-three stamps from twenty-five foreign countries commemorating fifty mathematicians, individuals who had contributed to mathematics, and mathematical topics, was presented. A mimeographed list of one hundred and twenty-eight stamps from twenty-six foreign countries was given to those present.

This list was a modified extension of those of Carl B. Boyer in his article, "Philately and Mathematics" (Scripta Mathematica **XV**(2): 105–114), and H. D. Larsen in "Mathematics on Stamps" (The Mathematics Teacher, 1955 (Nov.): 477–480).

Tabulation of the Wigner 9j-Coefficients. J. W. STEPHENSON AND K. SMITH. Tables of the Wigner 9j-coefficient, used in calculation of nuclear cross sections, have been calculated over the ranges $\frac{1}{2} \leq$ a, b, c, d, $\leq \frac{7}{2}$ and $0 \leq$ e, f, g, h, k ≤ 7. The calculation was performed on the IBM-650 digital computing machine at N. C. State College. The programming of this problem for the IBM 650 was explained.

A Study of Perturbation Methods in Nuclear Reactors. E. RUDOLPH PRINCE, JR. A mathematical investigation has been made concerning a new approach to nuclear reactor perturbation theory developed by Murray and Banister. A com-

parison was made with the perturbation theory as developed by Weinberg. This paper demonstrates that only a slight disparity exists between the solutions for the flux distribution for one group theory, although superficially the solutions appear to be quite different. Utilizing the new theory of Murray and Banister, the critical condition for the finite cylindrical reactor has been found.

Computer Solutions for Nuclear Reactor Kinetics Problems. ALBERT THOMSON MCMAIN, JR. A set of coupled differential equations with variable coefficients are presented as the mathematical model describing a nuclear reactor system. The kinetic response is studied by imposing various boundary and initial conditions and solving the equations numerically. The procedure is discussed and high-speed computer solutions for the response of reactor systems using this technique are reviewed. Effects of delayed neutrons and temperature on the kinetic response are presented graphically for comparison with the purely nuclear response. The accuracy of the numerical solutions is discussed and the generality and limitations of the mathematical model are pointed out.

Carbon Dioxide Fixation by the Oyster. CARL S. HAMMEN. Previous studies indicated that the mantle of the oyster has an active enzymatic role in handling carbon dioxide destined to form shell carbonate. Specimens of the oyster *Crassostrea virginica* in sea water to which carbon-14 labelled bicarbonate was added incorporated the radioactive carbon into the shell in eight out of ten cases. The amount of radioactivity was 26 to 143 times that of isolated shells kept in the same medium. Analysis of these shells by acid digestion into protein and carbonate fractions indicated that carbon dioxide of the medium entered both fractions, the carbonate in proportion to the measured activity of whole shells, and the protein in exceedingly variable amounts.

Isolated mantle tissue of the oyster, when incubated at 23°C. with labelled bicarbonate, fixed carbon dioxide into three general groups of organic compounds, two separable on the basis of ether and aqueous ethanol solubility, and a third consisting primarily of precipitated proteins. Fixation was detectable after six minutes of incubation. The first two classes of compounds were maximally labelled after about sixty minutes, while the protein fraction showed increased incorporation of carbon-14 up to five hours when it had the highest specific activity of the three fractions.

The Diagenetic Efficiency of Some Arctic Lakes. D. A. LIVINGSTONE. The poor preservation of microfossils in the sediments of several lakes in the Brooks Range, Alaska, indicates a rather high diagenetic efficiency. This impression can be supported by comparing the current rate of carbon fixation in northern Alaskan lakes with the rate at which fixed carbon has been deposited in the sediments during post-glacial time. The comparison indicates a diagenetic efficiency of ninety per cent in shallow ponds. Mineralization in deep lakes is probably more severe.

Some Measurements of Diurnal Oxygen Metabolism in Streams. CHARLES M. HOSKIN. Estimates of gross primary productivity and respiration rates have been

calculated from some North Carolina streams using diurnal oxygen metabolism data. The productivity/respiration ratio of each of twelve streams studied was considerably less than one, which indicated that the stream biota was dependent upon external organic matter as an energy source.

Use of Antibiotics for Obtaining Bacteria-Free Cultures of Arcella. J. E. DAVIS, JR. Penicillin, dihydrostreptomycin and achromycin were added both singly and in combinations to clone cultures of *Arcella* in attempts to obtain bacteria-free cultures. These antibiotics used singly or in combinations of any two did not render the cultures bacteria-free. Pure (bacteria-free) cultures were obtained only by the use of penicillin (800 units/ml.), dihydrostreptomycin (10 mg./ml.) and achromycin (100 mg./ml.) in combination.

In Vitro *Studies of Active Sodium and Potassium Transport in Mouse Ascites Tumor Cells.* EDGAR F. STILLWELL. The maintenance of low intracellular sodium and high intracellular potassium levels against steep electrochemical gradients requires the utilization of energy provided by the normal cellular metabolism. In metabolically depressed cells a leakage of K and a gain in Na is to be expected. In this study the effect of low-temperature, certain metabolic inhibitors and x-irradiation on intracellular Na and K levels in ascites tumor cells has been studied.

Ascites tumor cells showed a leakage of K and a gain in Na when maintained at 0°C. in Krebs-Ringer phosphate solution. When the cells were returned to body temperature approximately normal Na and K levels were re-established. DNP, NaCN, and IAA at a concentration of 2.5 mM. per liter caused K loss and Na gain at body temperature. However, NaF at concentrations of 2.5 and 8.2 mM. per liter was ineffective. DNP, NaCN, and IAA also inhibited the recovery or normal cation levels in low-temperature treated cells when returned to body temperature. NaF did not inhibit the recovery. Ascites tumor cells lost K and gained Na when exposed to from 10 to 50 Kr. of x-ray. Following the exposure at room temperature, irradiated cells showed a greater radiation effect at low-temperature than similar cells incubated at body temperature.

The Cytotoxicity of an Antiserum Prepared Against the Supernatant Fraction of a Cell.[5] EDWARD C. HORN AND MARY ELIZABETH HOUSE. A rabbit antiserum (AS) against the whole non-particulate supernatant fraction (soluble phase) of Ehrlich ascites tumor cells has been prepared which yields low tumor cell agglutination and moderate complement fixation titers completely comparable to those reported earlier for antisera prepared to other components of this same cell. In addition, the AS exhibited the cytotoxic properties previously demonstrated only by antisera to the whole nuclei and mitochondrial fraction of this cell. Again cytotoxicity could not have been predicted from the agglutination and CF tests.

The cytotoxic effect was further analyzed and found to be practically complement-independent. Cytotoxicity could be demonstrated by *in vivo* as well as *in*

[5] This work was supported in part by research grant RG-4203 from the National Institutes of Health, Public Health Service.

vitro exposure of the tumor cells to AS. The effects through *in vitro* exposure of the cells to the AS were found to vary with cell concentration exposed.

Effects of Depletion and Substitution of Perivesicular Mesenchyme upon the Development of the Thyroid Primordium. WILLIAM E. DOSSEL. The avian thyroid gland consists of two discrete lobes of tissue constructed of stromal and parenchymal elements derived, respectively, from the mesenchymal and entodermal tissues of the median ventral region of the embryonic pharynx. The question may be asked whether normal development requires an interaction of an inductive nature between the two tissues.

If the thyroid vesicle of a normal chick, three and a half days old, together with a quantity of tissue sufficiently large to include portions of the third and fourth visceral arches, is put into the coelom of a host of similar age, and allowed to develop to a total incubation age of eleven days, thyroid tissue develops from it that is histologically and functionally comparable with the thyroid glands of normal embryos of a like age. The tissue occurs as a discrete unit having a mass consistently less than the aggregate mass of the thyroid lobes of central embryos, but greater than that of either individual lobe. Depletion of the perivesicular mesenchyme, until only the cells immediate to the vesicle remain, does not materially affect the histogenesis or functional activity of the tissue, although the total amount of thyroid tissue in the implant is reduced as compared with the two previously described sorts of implants. If the vesicle is entirely denuded, through the use of tetrasodium versenate, and, after being embedded in mesenchymal blocks taken from the visceral arches or from the notochordal region, placed, together with its foreign mesenchyme, into the coelom, the thyroid tissue that is recovered after seven and one-half days' further incubation is recognizably thyroid, although with certain differences: first, the quantity of tissue is even less than that recovered from implants of the type previously described; second, the quantity of colloid is diminished even though the tissue is highly vascularized; third, the endothelial cells, that are found in the sinusoids that ramify throughout the tissue, are materially reduced in number.

It is suggested that, after the establishment of the definitive vesicle, the perivesicular mesenchyme plays a relatively non-specific role, that foreign mesenchyme cannot entirely supplant, in the subsequent histogenesis and attendant disposition for function of the entodermally-derived parenchymal component of the aggregate tissue. Certainly, if an interaction of an inductive nature occurs between the presumptive stromal and parenchymal elements, it has occurred prior to the time of the appearance of the definitive thyroid vesicle.

An Immuno-Histological Investigation of Tissue Antigens in the Frog Embryo. WILLIAM M. CLARKE. Antigenic substances have been associated with many events in embryonic differentiation, though this need not be in their capacity as antigens but may be due to some other biological activity.

The present study was undertaken to determine the localization of antigens in the tail bud stage of the frog and to correlate these localizations with certain cytotoxic effects of antisera to these antigens.

Mature ovarian oocytes and hatched tail buds of the frog, *Rana pipiens*, were homogenized and saline soluble extracts obtained. Rabbits immunized with these fractions produced antisera which were analyzed in interface precipitin tubes and serum agar diffusion plates. The anti-tail bud sera were shown to possess cytotoxic activity.

Globulin fractions of these antisera were conjugated with fluorescein isocyanate and employed as antigen specific histochemical stains on tissue sections. Using suitable optics, specific fluorescence was observed in the epithelial gland cells, lens anlage, epiphysis, cerebrospinal fluid and ependymal layer of the neural tube, and in certain cells of the notochord. Therefore, it is suggested that the epidermal cytolysis, neural abnormalities, and failure of eye development observed in the embryo cultures may be due to the action of antisera on these tissues disrupting the normal functions of antigenic substances in development.

Maternal antigens in mature frog oocytes as demonstrated by fluorescent antibody. LAUREL E. GLASS. The gamma globulin fractions from rabbit antisera against adult female frog blood components were conjugated with fluorescein isocyanate. Reactions with cellular antigens of the mature frog oocyte were localized by placing the tagged antisera on frozen or paraffin-embedded ovarian sections. After incubation, the excess antiserum was washed from the section and microscopic observations of the site of the antigen-antibody reactions were made using a mercury arc light source and an appropriate filter system. Antigens with combining groups similar to or identical with those found in maternal frog serum are localized in the following positions in the mature oocyte and associated ovarian cells of *Rana pipiens:* (1) in the cytoplasm of the cells of the theca interna, of the follicle layer, and of the oocyte cortex; (2) in a perinuclear zone, sometimes appearing here as a continuous finely granular band, sometimes as discontinuous clumps, and particularly present in large juxtanuclear bodies which may or may not be homologous to the Golgi complex; (3) in many intranuclear nucleoli; and (4) strongly localized as rims around large yolk platelets and generally strongly apparent in the vegetal regions of the egg. Recognizing the complexity of the antibody system used and of the genetic constitution of the oocyte, it was suggested that, even so, these data might have pertinence to current concepts of macromolecular transfer from maternal organism to oocyte or fetus.

A Cytological Analysis of the Salamander Cross, Triturus viridescens × *Taricha (Triturus) granulosa.*[6] H. E. LEHMAN AND LILLIAN M. YOUNGS. Reciprocal cross-fertilizations between these species have been obtained by following standard procedures for artificial fertilization of uterine eggs with "dry" sperm of the foreign species. Sperm entry and activation occurred in both crosses and the developmental fates of these hybrids were as follows.

Taricha granulosa ♀ × *T. viridescens* ♂ hybrids without exception were delayed approximately six hours before cleaving. Cleavage furrows usually were

[6] Supported by Grant RG-4193 from the National Institutes of Health, Public Health Service (1954–1957).

irregular or abortive, and most eggs cytolized during early division stages. One case developed into a partial blastula. Tissues from this embryo, when grafted to normal *Amblystoma punctatum* tailbud hosts, survived until donor controls were early tailbud embryos; however, the hybrid tissue stopped dividing and cytolized without visible differentiation. These data suggest that this cross may be inherently lethal during pre-gastrula stages of development.

Triturus viridescens ♀ × *T. granulosa* ♂ hybrids following fertilization maintained a developmental rate during cleavage and later embryonic stages that was entirely normal and characteristic of the maternal species. Approximately ten per cent of the cases were carried through metamorphosis and exhibited an intermediate condition between the parental controls with regard to pigmentation, skin glands, and external morphology. Chromosome counts from tail-tip preparations showed that they were eudiploid. It can be assumed from the cytological and morphological evidence that these individuals were true hybrids and capable of total development. The remainder of the hybrids were arrested at various periods in development from cleavage through tailbud stages. Transplantation of tissues from arrested hybrids to normal *A. punctatum* hosts revealed that the arrested hybrid cells, although capable of healing and of neural induction, cytolized without histological differentiation. Aceto-orcein chromosome smears of arrested tissues indicated that many of these embryos were hypodiploid mosaics. Their lethality probably stems from a failure of paternal chromosomes to be incorporated completely into the division figure during early cleavage stages.

A Preliminary Report on Extrinsic Factors Influencing Pigment Cell Morphology.[6] H. E. LEHMAN AND LILLIAN M. YOUNGS. It is generally held that amphibian pigment cell shape is determined by the genetic constitution of the individual cells in question. However, when transplantations of pigment anlagen (neural crest) are made between embryos of various species of salamanders, the phenotypic characteristics of donor pigment cells in a foreign environment may be strongly modified.

Preliminary experiments involving transplantation and injection indicate that these variations in genetic expression are correlated with species differences in the amount of the hormone intermedin released by the pituitary, this hormone having the well known capacity to cause pigment cell expansion.

Regional differences in the surface properties of the ectoderm-mesoderm terrain upon which pigment cells migrate and differentiate are being investigated through the use of tissue culture and time-lapse motion picture techniques. Attempts are being made to correlate observed pigment cell behavior and morphology *in vivo* with pigment cells in culture growing on various types of embryonic tissues or on different types of physical substrates (i.e., on glass, agar, water, silicone, celloidin, or hydrocarbon surfaces). At present it is possible only to say that specific substrates can determine cell shape to a greater or lesser degree, but as yet the observations *in vitro* do not provide a basis for explaining pigment cell morphology in normal development.

INTERVALS FOR THE CHARACTERISTIC ROOTS OF AN HERMITIAN MATRIX

By Alfred Brauer[1] and A. C. Mewborn[2]

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Recently Ky Fan [9] obtained lower and upper bounds for the different characteristic roots of an Hermitian matrix. More exactly, he proved the following theorem.

Theorem A. Let $A = (a_{ij})$ be an Hermitian matrix of order n with characteristic roots $\lambda_1 \leq \lambda_2 \leq \cdots \leq \lambda_n$. If $c_2, c_3, \cdots, c_n$ are positive numbers and $d_1, d_2, \cdots, d_n$ is an increasing sequence of real numbers such that

$$d_i - d_{i-1} \leqq \frac{1}{c_i} \qquad (i = 2, 3, \cdots, n)$$

and

$$a_{11} \leq d_1$$
$$a_{ii} + c_i \sum_{j<i} |a_{ij}|^2 \leq d_i \qquad (i = 2, 3, \cdots, n).$$

Then for each i

$$\lambda_i \leq d_i \qquad (i = 1, 2, \cdots, n).$$

Since the intervals obtained for the roots by this interesting result depend upon the choice of the constants c_i and d_i and on the ordering of the rows and columns of the given matrix, it may become difficult to use it for numerically given matrices of higher order.

Ky Fan's proof is based on the Separation Theorem for the characteristic roots of an Hermitian matrix A, first proved by Cauchy [8]:

> Between two consecutive characteristic roots of A there lies a root of each of the principal minors of order $n - 1$ of A.

E. T. Browne [7] studied the case where A has multiple roots more exactly and introduced the name "Separation Theorem."

Using the Separation Theorem a very simple method will be obtained in this paper which gives intervals for each of the n characteristic roots of A. In their simplest form these results can be formulated as follows.

Theorem 1. Let $\lambda_1 \leq \lambda_2 \leq \cdots \leq \lambda_n$ be the characteristic roots of an Hermitian matrix A. Let P be an arbitrary principal minor of order ν of A and U_ν

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[2] National Science Foundation fellow.

be the maximum of the row sums of the absolute values of the elements of P. Then

$$-U_{n-\nu+1} \leq \lambda_\nu \leq U_\nu \qquad (\nu = 1, 2, \cdots, n).$$

This result will be improved by using better bounds for the greatest and smallest roots of the principal minors of A. In particular, such new bounds will be obtained by bordering these minors in such a way that the matrices so obtained are generalized stochastic Hermitian matrices, and then by using the results of the first author on such matrices [3].

Instead of proving Theorem 1 we prove the following two theorems which contain Theorem 1 as a special case.

Theorem 2. If G_ν is an upper bound for the ν^{th} root of the Hermitian matrix A, then G_ν is also an upper bound for the ν^{th} root of any Hermitian matrix B which contains A as a principal minor.

Proof: Let m be the order of B and $\kappa_1 \leq \kappa_2 \leq \cdots \leq \kappa_m$ the characteristic roots of B. It follows from the Separation Theorem that κ_ν is less than or equal to the ν^{th} root of any principal minor of B of order $m - 1$, hence of any principal minor of order $m - 2$. Successive application of the Separation Theorem shows that $\kappa_\nu \leq \lambda_\nu$. This proves Theorem 2.

Using Theorem 2 we easily obtain upper bounds for all the characteristic roots of any Hermitian matrix. It follows that the smallest root of any Hermitian matrix is less than or equal to the smallest main diagonal element, a well known result. Since the roots of a matrix of order 2 can be easily obtained, we obtain at once a better result; namely, that the smallest root of an Hermitian matrix is less than or equal to the smallest root of any principal minor of order 2. Moreover, the second root is less than or equal to the minimum of the greatest roots of all principal minors of order 2.

In particular we now have upper bounds for the two smaller roots of any principal minor of order 3. For the largest root of such a minor we may take as upper bound the greatest sum of absolute values of the elements of one row or a better bound which may be obtained by one of the well known methods [1, 4, 10, 11, and 12]. Hence, we obtain bounds for the three smallest roots of the matrix A. In this way we may continue. If we have upper bounds for all the roots of a principal minor of order ν, then we have bounds for the first ν roots of any minor of order $\nu + 1$ which contains this minor as principal minor. Taking for the largest root of this minor of order $\nu + 1$ one of the bounds for the largest root, we obtain by induction upper bounds for all the roots of A.

In a very similar way we obtain

Theorem 3. If L_ν is a lower bound for the ν^{th} root of any Hermitian matrix A of order n, then it is also a lower bound for the $\nu + \tau^{\text{th}}$ root of any Hermitian matrix B of order $\nu + \tau$ which contains A as a principal minor.

Proof: The $\nu + 1^{\text{th}}$ root of any Hermitian matrix of order $\nu + 1$ which contains A as a principal minor is greater than or equal to the ν^{th} root of A. Continuing in this way, the theorem is obtained.

A special case of Theorem 3 is the well known fact that the greatest root of an

Hermitian matrix is greater than or equal to the greatest main diagonal element. Since the greatest root of each minor of order 2 can be easily determined, we obtain from Theorem 2 the better result that λ_n is greater than or equal to the largest characteristic root of any minor of order 2. Moreover, λ_{n-1} is greater than or equal to the maximum of the smaller roots of all principal minors of order 2. Since it is easy to obtain a lower bound for the smallest root of any minor, it follows as above that we get lower bounds for all the roots of an Hermitian matrix A.

Often the obtained bounds can be improved by using the trace of A or A^2.

Let us illustrate our method with an example. We choose an arbitrary symmetric matrix of order 4.

$$A = \begin{pmatrix} 12 & 13 & 9 & 7 \\ 13 & 14 & 14 & 12 \\ 9 & 14 & 11 & 13 \\ 7 & 12 & 13 & 10 \end{pmatrix}.$$

Since the elements are not too small we cannot expect that the intervals for the roots obtained by our methods will be very small. It is easy to see that the smallest root of any principal minor of order 2 is $\frac{1}{2}[21 - \sqrt{677}]$. Therefore, by Theorem 2,

$$\lambda_1 \leq \tfrac{1}{2}[21 - \sqrt{677}]. \tag{1}$$

Similarly, the smallest of the larger roots of principal minors of order 2 is $11 + 5\sqrt{2}$. Hence

$$\lambda_2 \leq 11 + 5\sqrt{2}. \tag{2}$$

Taking the principal minor of order 3 obtained by omitting the second row and second column, which has 33 as greatest row sum, it follows at once that

$$\lambda_3 < 33. \tag{3}$$

We have strict inequality since 33 is not a common point of all the circles. On the other hand, the greatest of the smaller roots of principal minors of order 2 is $11 - 5\sqrt{2}$. Hence

$$\lambda_3 \geq 11 - 5\sqrt{2}. \tag{4}$$

The smallest root of the principal minor obtained by omitting the second row and column is greater than -11. Hence,

$$\lambda_2 > -11. \tag{5}$$

The smallest root of the matrix is obviously greater than -25.

These bounds were obtained using only the well-known circles whose centers are the main diagonal elements and which contain the characteristic roots. If we use instead of these the ovals of Cassini, whose foci are the main diagonal elements, we can improve the lower bound for λ_1 and λ_2 and the upper bounds for λ_3. We obtain

$$\lambda_1 > -23.5$$

(6) $$\lambda_2 > -9.9$$

$$\lambda_3 < 31.5.$$

To improve these bounds furthermore, we can use the following method. Any Hermitian matrix can be bordered in many different ways such that the matrix so obtained is Hermitian and generalized stochastic. Using now a theorem of [3] we can subtract from all the elements of each column an arbitrarily chosen number without changing the non-trivial characteristic roots of the stochastic matrix. In this way we may get good bounds for these roots, and, since the roots of the considered minor separate the roots of the bordered matrix, we often obtain better bounds for the roots of the minor. We explain this method by our previous example.

We border the minor A_{11} of the given matrix obtaining the generalized stochastic matrix

$$C_{11} = \begin{pmatrix} -7 & 20 & 22 & 25 \\ 20 & 14 & 14 & 12 \\ 22 & 14 & 11 & 13 \\ 25 & 12 & 13 & 10 \end{pmatrix}$$

with row sums 60. We subtract from the columns 22, 14, 14, 13, obtaining the generalized stochastic matrix

$$D_{11} = \begin{pmatrix} -29 & 6 & 8 & 12 \\ -2 & 0 & 0 & -1 \\ 0 & 0 & -3 & 0 \\ 3 & -2 & -1 & -3 \end{pmatrix}.$$

The non-trivial roots $\rho_1 \leq \rho_2 \leq \rho_3$ of this matrix are the roots of the principal minor

(7) $$\begin{pmatrix} -29 & 6 & 12 \\ -2 & 0 & -1 \\ 3 & -2 & -3 \end{pmatrix}.$$

Hence the roots of C_{11} are ρ_1, ρ_2, ρ_3 and 60. It follows by using the circles for the rows of (7) that 60 is the largest root of C_{11}. Multiplying the first row of (7) by 2, the third row by $\frac{4}{3}$, and dividing the first and third columns, respectively, by 2 and $\frac{4}{3}$, we obtain the matrix

$$\begin{pmatrix} -29 & 12 & 18 \\ -1 & 0 & -\frac{3}{4} \\ 2 & -\frac{8}{3} & -3 \end{pmatrix}$$

whose greatest root is less than $\frac{7}{4}$. Hence by Theorems 2 and 3

(8) $$\lambda_3 \leq \rho_3 < \frac{7}{4},$$

improving (2) considerably.

Now we border A_{11} in a different way obtaining the matrix

$$C'_{11} = \begin{pmatrix} 13 & 10 & 12 & 15 \\ 10 & 14 & 14 & 12 \\ 12 & 14 & 11 & 13 \\ 15 & 12 & 13 & 10 \end{pmatrix}$$

with the roots $\rho'_1 \leq \rho'_2 \leq \rho'_3 \leq 50$. Subtracting 13, 12, 12, and 12 from the columns of C_{11}, we obtain the matrix

$$D'_{11} = \begin{pmatrix} 0 & -2 & 0 & 3 \\ -3 & 2 & 2 & 0 \\ -1 & 2 & -1 & 1 \\ 2 & 0 & 1 & -2 \end{pmatrix}$$

having the roots ρ'_1, ρ'_2, ρ'_3 and 1.

Since the smallest root of A_{11} is greater than or equal to ρ'_1, we obtain, using the circles,

$$\lambda_2 \geq \rho'_1 > -5 \tag{9}$$

improving the second inequality (6).

We now border the given matrix A obtaining

$$C = \begin{pmatrix} 60 & 0 & -12 & -6 & -1 \\ 0 & 12 & 13 & 9 & 7 \\ -12 & 13 & 14 & 14 & 12 \\ -6 & 9 & 14 & 11 & 13 \\ -1 & 7 & 12 & 13 & 10 \end{pmatrix}$$

with the roots $\sigma_1 \leq \sigma_2 \leq \sigma_3 \leq \sigma_4$ and the trivial root 41. Adding 6 to the first column and subtracting 11, 12, 12, and 9 from the other columns of C we obtain the matrix

$$D = \begin{pmatrix} 66 & -11 & -24 & -18 & -10 \\ 6 & 1 & 1 & -3 & -2 \\ -6 & 2 & 2 & 2 & 3 \\ 0 & -2 & 2 & -1 & 4 \\ 5 & -4 & 0 & 1 & 1 \end{pmatrix}$$

with roots σ_1, σ_2, σ_3, σ_4 and 3. Using the circles for the matrix D we get

$$\lambda_1 \geq \sigma_1 > -11 \tag{10}$$

which improves the first inequality (6).

We now border the matrix A in a different way obtaining the matrix

$$C' = \begin{pmatrix} 6 & 18 & 6 & 12 & 17 \\ 18 & 12 & 13 & 9 & 7 \\ 6 & 13 & 14 & 14 & 12 \\ 12 & 9 & 14 & 11 & 13 \\ 17 & 7 & 12 & 13 & 10 \end{pmatrix}$$

with roots $\sigma_1' \leq \sigma_2' \leq \sigma_3' \leq \sigma_4' \leq 59$. Subtracting 14, 13, 13, 12 and 12 from the columns of C' we obtain the matrix

$$D' = \begin{pmatrix} -8 & 5 & -7 & 0 & 5 \\ 4 & -1 & 0 & -3 & -5 \\ -8 & 0 & 1 & 2 & 0 \\ -2 & -4 & 1 & -1 & 1 \\ 3 & -6 & -1 & 1 & -2 \end{pmatrix}$$

with roots σ_1', σ_2', σ_3', σ_4' and -5. Applying the circles we obtain at once

$$\lambda_3 \leq \sigma_4' < 11 \tag{11}$$

improving the third inequality (6).

Instead of bordering such that a stochastic matrix is obtained it may be sometimes better to border such that the new matrix has a given characteristic vector and then use theorem 27 of [3].

Some of the preceding results could be improved further by refining the used methods. Later we will get better estimates for some of these roots.

The largest root λ_4 is the largest root of a positive matrix and can be computed as exactly as desired by using the method of the paper [6]. We multiply the rows of A by $\frac{32}{25}$, $\frac{300}{301}$, $\frac{48}{43}$, and $\frac{683}{550}$, respectively, obtaining the matrix

$$\begin{pmatrix} 12 & \frac{31,304}{1,875} & \frac{258}{25} & \frac{4,928}{683} \\ \frac{24,375}{2,408} & 14 & \frac{25}{2} & \frac{1,980,000}{205,583} \\ \frac{675}{86} & \frac{392}{25} & 11 & \frac{343,200}{29,369} \\ \frac{4,781}{704} & \frac{205,583}{13,750} & \frac{381,797}{26,400} & 10 \end{pmatrix}.$$

The largest root lies between the smallest and largest row sums,

$$46.2046 < \lambda_4 < 46.2537. \tag{12}$$

These bounds could be improved as much as desired. Using (12) we can now improve our other bounds.

The trace of A is 47. The trace of A^2 is $443 + 705 + 567 + 462 = 2177$. Hence

$$\lambda_1 + \lambda_2 + \lambda_3 + \lambda_4 = 47,$$
$$\lambda_1^2 + \lambda_2^2 + \lambda_3^2 + \lambda_4^2 = 2177,$$

and by (12)

$$.7463 < \lambda_1 + \lambda_2 + \lambda_3 < .7954 \tag{13}$$

(14) $$2134.8650 < \lambda_4^2 < 2139.4048$$

(15) $$37.5952 < \lambda_1^2 + \lambda_2^2 + \lambda_3^2 < 42.1350.$$

Since by (4),

(16) $$\lambda_3^2 \geq 171 - 110\sqrt{2} > 15.4270,$$

it follows from (15) that

(17) $$\lambda_1^2 < 42.1350 - 15.4270 = 26.7080$$
$$\lambda_1 > -\sqrt{26.7080}.$$

From (13)

$$\lambda_1 + \lambda_2 < .7954 - \lambda_3$$

and, since $\lambda_3 > .7954$

(18) $$\lambda_1^2 + \lambda_2^2 + 2\lambda_1\lambda_2 > (.7954)^2 - 1.5908\lambda_3 + \lambda_3^2.$$

From (15)

(19) $$\lambda_1^2 + \lambda_2^2 < 42.1350 - \lambda_3^2.$$

Multiplying (19) by 2 and subtracting (18) we get

$$(\lambda_1 - \lambda_2)^2 < 83.6374 + 1.5908\lambda_3 - 3\lambda_3^2$$

and so

$$3\lambda_3^2 - 1.5908\lambda_3 - 83.6375 < 0.$$

This implies

(20) $$\lambda_3 < 5.5519.$$

Since by (1)

$$\lambda_1 < -2.5079$$

it follows from (13) and (20) that

(21) $$\lambda_2 > .7463 + 2.5079 - 5.5519 = -2.2977.$$

Combining now (1), (4), (8), (12), (17), (20) and (21), we obtain the following inequalities for the characteristic roots of A,

$$-5.1680 < \lambda_1 < -2.5079$$
$$-2.2977 < \lambda_2 < 1.75$$
$$3.9285 < \lambda_3 < 5.5519$$
$$46.2046 < \lambda_4 < 46.2537.$$

A complete separation of the roots is obtained. These bounds can be improved further by computing λ_4 more exactly. It follows from [6] that this root can be computed as exactly as desired, and better bounds for λ_4 give better bounds for

the other roots. Moreover, we can consider the minors of the matrix A^2 or those of other polynomials in A.

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AN OPTIMUM EXPLICIT RECURRENCE FORMULA FOR THE DIFFUSION EQUATION

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Consider the one-dimensional diffusion equation,

$$L[\varphi(x, t)] = \varphi_t - \varphi_{xx} = 0. \tag{1}$$

Perhaps the best known difference approximation to (1) is

$$N[\Phi_{m,n}] = \frac{\Phi_{m,n+1} - \Phi_{m,n}}{\Delta t} - \frac{1}{(\Delta x)^2}(\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n}) = 0. \tag{2}$$

If $\varphi(x, t)$ is a sufficiently differentiable solution of (1), then it is easy to show, by using an extended form of the mean value theorem and the equation (1) itself, that

$$(3)\qquad N[\varphi(x,t)] = \left[\frac{\Delta t}{2} - \frac{(\Delta x)^2}{12}\right]\varphi_{xxt} + \Theta[(\Delta t)^2, (\Delta x)^4] = \Theta[(\Delta t), (\Delta x)^2],$$

and this is called the truncation error in (1). Obviously, we can approximate to the first term in the truncation error as follows

$$(4)\qquad \left[\frac{\Delta t}{2} - \frac{(\Delta x)^2}{12}\right]\varphi_{xxt} \approx \left[\frac{1}{2(\Delta x)^2} - \frac{1}{12(\Delta t)}\right] \cdot [(\varphi_{m+1,n+1} - 2\varphi_{m,n+1} + \varphi_{m-1,n+1}) - (\varphi_{m+1,n} - 2\varphi_{m,n} + \varphi_{m-1,n})].$$

Substituting $\Phi_{m,n}$ for $\varphi_{m,n}$ in the right hand side of the above approximation and subtracting the resulting expression from $N[\Phi_{m,n}]$, we obtain a new difference operator defined as follows:

$$M[\Phi_{m,n}] = N[\Phi_{m,n}] - \left[\frac{1}{2(\Delta x)^2} - \frac{1}{12(\Delta t)}\right] \cdot [(\Phi_{m+1,n+1} - 2\Phi_{m,n+1} + \Phi_{m-1,n+1}) - (\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n})].$$

Proceeding as we did in the derivation of (3), we find that the truncation error in the equation

$$M[\Phi_{m,n}] = 0$$

is only $\Theta[(\Delta t)^2, (\Delta x)^4]$. Combining like terms, and letting $r = \dfrac{(\Delta t)}{(\Delta x)^2}$, the above equation can be put in the form

$$(5)\qquad \begin{aligned}\Phi_{m,n+1} - \Phi_{m,n} &= \left(\frac{r}{2} + \frac{1}{12}\right)(\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n}) \\ &\quad + \left(\frac{r}{2} - \frac{1}{12}\right)\cdot(\Phi_{m+1,n+1} - 2\Phi_{m,n+1} + \Phi_{m-1,n+1})\end{aligned}$$

which was given by Douglas [1]. It has the great virtue of being stable for all mesh shapes. Crandall, who published a special case of (5), called it an optimum implicit recurrence formula for the heat equation [2]. It is the purpose of this note to show how a slight variation in the procedure which we followed to obtain (5) leads to what might be called an optimum explicit recurrence formula for (1).

The derivation of the formula which we have in mind branches off from our derivation of (5) between equation (3) and approximation (4). In (4), the time derivative in φ_{xxt} was approximated by a forward time difference. Choosing now a backward time difference, we get

$$(6)\qquad \left[\frac{\Delta t}{2} - \frac{(\Delta x)^2}{12}\right]\varphi_{xxt} \approx \left[\frac{1}{2} - \frac{(\Delta x)^2}{12(\Delta t)}\right] \cdot \left[\frac{\varphi_{m+1,n} - 2\varphi_{m,n} + \varphi_{m-1,n}}{(\Delta x)^2} - \frac{\varphi_{m+1,n-1} - 2\varphi_{m,n-1} + \varphi_{m-1,n-1}}{(\Delta x)^2}\right].$$

This leads to the improved difference operator

$$Q[\Phi_{m,n}] = N[\Phi_{m,n}] - \left[\frac{1}{2(\Delta x)^2} - \frac{1}{12(\Delta t)}\right] \cdot [(\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n}) - (\Phi_{m+1,n-1} - 2\Phi_{m,n-1} + \Phi_{m-1,n-1})].$$

The equation $Q[\Phi_{m,n}] = 0$, which can be put in the form

$$\frac{\Phi_{m,n+1} - \Phi_{m,n}}{\Delta t} = \left[\frac{3}{2} - \frac{(\Delta x)^2}{12(\Delta t)}\right]\left(\frac{\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n}}{(\Delta x)^2}\right) - \left[\frac{1}{2} - \frac{(\Delta x)^2}{12(\Delta t)}\right]\left(\frac{\Phi_{m+1,n-1} - 2\Phi_{m,n-1} + \Phi_{m-1,n-1}}{(\Delta x)^2}\right) \tag{7}$$

has a truncation error which is $\Theta[(\Delta t)^2, (\Delta x)^4]$. Letting $r = \dfrac{\Delta t}{(\Delta x)^2}$ again, the above equation can be written

$$(\Phi_{m,n+1} - \Phi_{m,n}) = \left(\frac{3r}{2} - \frac{1}{12}\right)(\Phi_{m+1,n} - 2\Phi_{m,n} + \Phi_{m-1,n}) - \left(\frac{r}{2} - \frac{1}{12}\right)(\Phi_{m+1,n-1} - 2\Phi_{m,n-1} + \Phi_{m-1,n-1}). \tag{8}$$

Applying the von Neumann stability test to this equation, we assume a solution of the form $\Phi_{m,n} = \beta^n e^{im\theta}$. Substitution in (8) leads to the following quadratic equation for β,

$$\beta^2 + \left[\left(6r - \frac{1}{3}\right)\sin^2\frac{\theta}{2} - 1\right]\beta - \left(2r - \frac{1}{3}\right)\sin^2\frac{\theta}{2} = 0. \tag{9}$$

Necessary and sufficient conditions that neither root of (9) lie outside the unit circle are the following inequalities:

$$\left|6r\sin^2\frac{\theta}{2} - \frac{1}{3}\sin^2\frac{\theta}{2} - 1\right| \leq 1 - 2r\sin^2\frac{\theta}{2} + \frac{1}{3}\sin^2\frac{\theta}{2} \leq 2. \tag{10}$$

The first of these can be written

$$\left|\left(1 - 2r\sin^2\frac{\theta}{2} + \frac{1}{3}\sin^2\frac{\theta}{2}\right) - 4r\sin^2\frac{\theta}{2}\right| \leq 1 - 2r\sin^2\frac{\theta}{2} + \frac{1}{3}\sin^2\frac{\theta}{2},$$

which is easily seen to be satisfied for

$$r \leq \frac{\csc^2\dfrac{\theta}{2} + \dfrac{1}{3}}{4}.$$

This will hold for all real θ if

$$r \leq \tfrac{1}{3}. \tag{11}$$

The second inequality of (10) is also easily seen to hold for all real θ provided r satisfies (11). Hence, (11) is the stability condition for equation (8).

In order to use equation (8), one must give special consideration to the problem of how to start the stepping ahead process from the line on which the initial data is given. One very satisfactory solution to this problem is to use the implicit Douglas formula (5) to take the first step and then to switch over to the explicit formula (8) for the rest of the computation. Since (5) and (8) have essentially

the same truncation error, it seems reasonable to hope that this combined use of the two formulas would yield results as accurate as those that would be obtained by using the Douglas implicit formula all the way. This hope has been justified by experiments which we have run on the North Carolina State College IBM 650.

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CONCERNING QUASI-HARMONIC OPERATORS[1]

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INTRODUCTION. In two previous papers ([1] and [2]) the author has extended H. S. Wall's theory [3] of *harmonic matrices*, the final extension including systems of Stieltjes mean-integral equations in which certain discontinuities may occur. Computation with these mean-integrals is described in detail in [2] (esp. section 3). We are here concerned with the reduction of such systems to a "second-order" form, and with a special case of this form which exhibits relations analogous to the *Euler formula*: $e^{ix} = \cos x + i \sin x$.

In a linear normed complete space S, we let $QH(S)$ denote the class of *quasi-harmonic operators* over S and $Q\Phi(S)$ the corresponding class of *generators, i.e.*, functions F from the real numbers to the space $B(S)$ of continuous linear transformations in S such that $F(0) = 0$, F is of bounded variation on each interval, and

$$[\Delta^- F(u)]^2 = [\Delta^+ F(u)]^2 = 0 \text{ for each real } u,$$

where $\Delta^- F(u) = F(u) - F(u-)$ and $\Delta^+ F(u) = F(u+) - F(u)$. We have established (Theorem 4.3 of [2]) a one-to-one correspondence between $QH(S)$ and $Q\Phi(S)$ which, for M in the former and F in the latter, is uniquely determined by each of the following formulas:

$$M(s, t) = {}_s\Pi^t\{1 + dF\} \tag{1}$$

$$M(s, t) = 1 + \int_s^t M(s, I) \cdot dF \tag{2}$$

$$M(s, t) = 1 + \int_s^t dF \cdot M(I, t) \tag{3}$$

[1] Presented to the American Mathematical Society on August 26, 1957.

$$F(u) = \int_0^u M(I, 0)\cdot dM(0, I) \tag{4}$$

$$F(u) = -\int_0^u dM(I, 0)\cdot M(0, I) \tag{5}$$

(here 1 denotes the identity in $B(S)$, and I the identity function on the real numbers). Let this correspondence be indicated by the functional notation: $M = \mathcal{E}(F)$.

THEOREM A. Suppose S is a linear normed complete space, each of F, G, and F-G belongs to $Q\Phi(S)$, $M = \mathcal{E}(F)$ and $N = \mathcal{E}(F - G)$. The formula,

$$K(u) = \int_0^u N(0, I)\cdot dG\cdot N(I, 0), \tag{6}$$

defines a member K of $Q\Phi(S)$ such that if $P = \mathcal{E}(K)$ then

$$M(s, t) = N(s, 0)P(s, t)N(0, t). \tag{7}$$

PROOF. Suppose K is defined by (6), and let $E = F - G$. Clearly K is of bounded variation on each interval and $K(0) = 0$. For each real u we have

$$\begin{aligned}\Delta^- K(u) &= \frac{N(0, u-) + N(0, u)}{2}\cdot \Delta^- G(u)\cdot \frac{N(u-, 0) + N(u, 0)}{2}\\ &= N(0, u)\,\frac{N(u, u-) + 1}{2}\cdot \Delta^- G(u)\cdot \frac{N(u-, u) + 1}{2}\,N(u, 0)\\ &= N(0, u)\{1 - \tfrac{1}{2}\Delta^- E(u)\}\cdot \Delta^- G(u)\cdot \{1 + \tfrac{1}{2}\Delta^- E(u)\}N(u, 0),\end{aligned}$$

and similarly

$$\Delta^+ K(u) = N(0, u)\{1 + \tfrac{1}{2}\Delta^+ E(u)\}\cdot \Delta^+ G(u)\cdot \{1 - \tfrac{1}{2}\Delta^+ E(u)\}N(u, 0).$$

Since each of E and G belongs to $Q\Phi(S)$ it follows that

$$[\Delta^- K(u)]^2 = [\Delta^+ K(u)]^2 = 0,$$

so that K belongs to $Q\Phi(S)$.

Now, by the characterization (3) of $\mathcal{E}$, we have the formula,

$$M(s, t) = 1 + \int_s^t dE\cdot M(I, t) + \int_s^t dG\cdot M(I, t),$$

which (by Theorem 4.2(i) of [2]) implies

$$M(s, t) = N(s, t) + \int_s^t N(s, I)\cdot dG\cdot M(I, t);$$

hence we have

$$N(0, s)M(s, t)N(t, 0) = 1 + \int_s^t N(0, I)\cdot dG\cdot M(I, t)N(t, 0).$$

With $P = \mathcal{E}(K)$ this will imply (7), provided that

$$\int_s^t N(0, I)\cdot dG\cdot M(I, t) = \int_s^t dK\cdot N(0, I)M(I, t).$$

By Corollary 3.3a of [2], it is sufficient to show that

$$\int_0^t dG \cdot M(I, 0) = \int_0^t dG \cdot N(I, 0) \cdot N(0, I) M(I, 0);$$

hence, by Corollary 3.4b of [2], we need only to show that if Δ denotes either Δ^- or Δ^+ then for each real u

$$\Delta G(u) \cdot \Delta N(u, 0) \cdot \Delta[N(0, u) M(u, 0)] = 0.$$

These relations are indeed fulfilled, as the following computations show:

$$\Delta^- N(u, 0) = N(u, 0) - N(u-, 0) = -\Delta^- E(u) \cdot N(u, 0),$$

$$\Delta^-[N(0, u) M(u, 0)] = N(0, u)[1 - N(u, u-) M(u-, u)] M(u, 0)$$

$$= -N(0, u)\{1 - \Delta^- E(u)\} \cdot \Delta^- G(u) \cdot M(u, 0),$$

$$\Delta^- N(u, 0) \cdot \Delta^-[N(0, u) M(u, 0)] = \Delta^- E(u) \cdot \Delta^- G(u) \cdot M(u, 0)$$

$$= -\Delta^- G(u) \cdot \Delta^- E(u) \cdot M(u, 0),$$

and, similarly,

$$\Delta^+ N(u, 0) = N(u+, 0) - N(u, 0) = -\Delta^+ E(u) \cdot N(u, 0),$$

$$\Delta^+[N(0, u) M(u, 0)] = N(0, u)[N(u, u+) M(u+, u) - 1] M(u, 0)$$

$$= -N(0, u)\{1 + \Delta^+ E(u)\} \cdot \Delta^+ G(u) \cdot M(u, 0),$$

$$\Delta^+ N(u, 0) \cdot \Delta^+[N(0, u) M(u, 0)] = \Delta^+ E(u) \cdot \Delta^+ G(u) \cdot M(u, 0)$$

$$= -\Delta^+ G(u) \cdot \Delta^+ E(u) \cdot M(u, 0).$$

This completes our proof of Theorem A.

THEOREM B. Suppose each of S_1 and S_2 is a linear normed complete space, F is a member of $Q\Phi(S_1 \times S_2)$ such that F_{11} is in $Q\Phi(S_1)$ and F_{22} is in $Q\Phi(S_2)$, and $M_* = \mathcal{E}(F)$ and $N_1 = \mathcal{E}(F_{11})$ and $N_2 = \mathcal{E}(F_{22})$. The formula

$$K(u) = \begin{pmatrix} 0 & \int_0^u N_1(0, I) \cdot dF_{12} \cdot N_2(I, 0) \\ \int_0^u N_2(0, I) \cdot dF_{21} \cdot N_1(I, 0) & 0 \end{pmatrix} \tag{8}$$

defines a member K of $Q\Phi(S_1 \times S_2)$ such that if $P = \mathcal{E}(K)$ then

$$M_{ij}(s, t) = N_i(s, 0) P_{ij}(s, t) N_j(0, t). \tag{9}$$

PROOF. It follows directly from the hypotheses concerning F that, if u is a real number and Δ denotes either Δ^- or Δ^+, the matrix

$$\begin{pmatrix} \Delta F_{12}(u) \cdot \Delta F_{21}(u) & \Delta F_{11}(u) \cdot \Delta F_{12}(u) + \Delta F_{12}(u) \cdot \Delta F_{22}(u) \\ \Delta F_{21}(u) \cdot \Delta F_{11}(u) + \Delta F_{22}(u) \cdot \Delta F_{21}(u) & \Delta F_{21}(u) \cdot \Delta F_{12}(u) \end{pmatrix}$$

represents the zero transformation in $B(S_1 \times S_2)$. Thus, with the identifications: $S = S_1 \times S_2$ and

$$G = \begin{pmatrix} 0 & F_{12} \\ F_{21} & 0 \end{pmatrix} \quad \text{and} \quad N = \begin{pmatrix} N_1 & 0 \\ 0 & N_2 \end{pmatrix},$$

we see that Theorem A is directly applicable here and Theorem B is proved.

THEOREM C. Suppose each of S_1 and S_2 is a linear normed complete space, T is a continuous linear transformation from S_1 into S_2, F is a member of

$$Q\Phi(S_1 \times S_2)$$

with the form

$$F(u) = \begin{pmatrix} 0 & W(u) \\ TW(u)T & 0 \end{pmatrix}, \tag{10}$$

and $M = \mathcal{E}(F)$. Then WT is in $Q\Phi(S_1)$ and

$$M_{11} \pm M_{12}T = \mathcal{E}(\pm WT), \qquad M_{22}T \pm M_{21} = T\mathcal{E}(\pm WT); \tag{11}$$

moreover, TW is in $Q\Phi(S_2)$ and

$$TM_{11} \pm M_{21} = \mathcal{E}(\pm TW)T, \qquad M_{22} \pm TM_{12} = \mathcal{E}(\pm TW). \tag{12}$$

PROOF. From (10) it follows that if Δ denotes either Δ^- or Δ^+ then

$$[\Delta F(u)]^2 = \begin{pmatrix} [\Delta W(u) \cdot T]^2 & 0 \\ 0 & [T \cdot \Delta W(u)]^2 \end{pmatrix};$$

thus one sees that WT is in $Q\Phi(S_1)$ and TW is in $Q\Phi(S_2)$. Using the characterization (2) of $\mathcal{E}$ one readily deduces the formulas:

$$M_{11}(s, t) \pm M_{12}(s, t)T = 1 \pm \int_s^t [M_{11}(s, I) \pm M_{12}(s, I)T] \cdot dWT$$

$$M_{22}(s, t)T \pm M_{21}(s, t) = T \pm \int_s^t [M_{22}(s, I)T \pm M_{21}(s, I)] \cdot dWT$$

By a minor variation of Theorem 4.2(ii) of [2], these formulas are found to imply (11). Using the characterization (3) of $\mathcal{E}$ and a similar variation of Theorem 4.2(i) of [2], one is led directly to (12) and Theorem C is proved.

REMARK. If, in Theorem C, S_1 is S_2 and T is the complex unit i, formulas (11) and (12) are equivalent and give the following "trigonometric" form for M:

$$M = \begin{pmatrix} \frac{1}{2}[\mathcal{E}(iW) + \mathcal{E}(-iW)] & \frac{1}{2i}[\mathcal{E}(iW) - \mathcal{E}(-iW)] \\ -\frac{1}{2i}[\mathcal{E}(iW) - \mathcal{E}(-iW)] & \frac{1}{2}[\mathcal{E}(iW) + \mathcal{E}(-iW)] \end{pmatrix}.$$

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A LIMIT THEOREM OF CRAMÉR AND ITS GENERALIZATIONS*

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We shall start with the following

Theorem 1. Let $\{\xi_n\}$ be a sequence of random variables, with the d.f.'s $\{F_n\}$. Suppose that $F_n(x)$ tends to a d.f. $F(x)$ as $n \to \infty$.

Let $\{\eta_n\}$ be another sequence of random variables, and suppose that η_n converges in probability to a constant c as $n \to \infty$. Put

$$X_n = \xi_n + \eta_n\,,\ Y_n = \xi_n\eta_n\,,\ Z_n = \xi_n/\eta_n\,.$$

Then the d.f. of X_n tends to $F(x - c)$ as $n \to \infty$. Further if $c > 0$, the d.f. of Y_n tends to $F\left(\frac{x}{c}\right)$, while the d.f. of Z_n tends to $F(cx)$ as $n \to \infty$. If $c < 0$, the d.f. of Y_n tends to $1 - F\left(\frac{x}{c}\right)$, while the d.f. of Z_n tends to $1 - F(cx)$ as $n \to \infty$.

This theorem is stated by Cramér in his famous text book [2] in §20.6 (p. 254) and is well known, being originally due to J. L. Doob [3]. D. Dugué [4] also proved the same results. Of course a partial result, i.e., the special case of X_n in which $c = 0$ had already been obtained by A. Kolmogorov in his famous booklet [5]. This means that the convergence in probability implies the convergence in law.

Cramér also states in the same book in §22.6 (p. 299) the following generalization of Theorem 1 and through the whole of this book he makes effective use of this theorem, but he skips its proof.

Theorem 2. Suppose that we have for every index $n = 1, 2, \cdots$

$$\mathbf{y}_n = A\mathbf{x}_n + \mathbf{z}_n$$

where $\mathbf{x}_n$, $\mathbf{y}_n$ and $\mathbf{z}_n$ are k-dimensional random vectors, while A is a matrix of order k with constant elements. Suppose further that, as $n \to \infty$, the k-dimensional distribution of $\mathbf{x}_n$ tends to a certain limiting distribution, while $\mathbf{z}_n$ converges in probability to zero. Then $\mathbf{y}_n$ has the limiting distribution defined by the linear transformation $\mathbf{y} = A\mathbf{x}$, where $\mathbf{x}$ has the limiting distribution of the $\{\mathbf{x}_n\}$.

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It seems to be necessary to prove this theorem rigorously and to pick out the points which make the proof go, in order to consider the further generalization of Theorem 1.

Proof of Theorem 2.

Let the d.f. of $\mathbf{x}_n$ be $F_n(x_1, \cdots, x_k)$ and its limiting d.f. be $F(x_1, \cdots, x_k)$ and further let the d.f. of $\mathbf{y}_n$ be $G_n(y_1, \cdots, y_k)$; then it will be sufficient to prove the following: For any k-dimensional interval

$$[\mathbf{a}; \mathbf{b}) \equiv \{\mathbf{x}; a_1 \leq x_1 < b_1, a_2 \leq x_2 < b_2, \cdots, a_k \leq x_k < b_k\}, \tag{1}$$

we shall define the difference as follows;

$$\begin{aligned}\Delta_k\Phi(\mathbf{a}; \mathbf{b}) = {} & \Phi(b_1, b_2, \cdots, b_k) - \Phi(a_1, b_2, \cdots, b_k) - \Phi(b_1, a_2, \cdots, b_k) \cdots - \\ & \Phi(b_1, b_2, \cdots, a_k) + \Phi(a_1, a_2, \cdots, b_k) + \Phi(a_1, b_2, a_3, \cdots, b_k) + \cdots \\ & + (-1)^k \Phi(a_1, a_2, \cdots, a_k);\end{aligned} \tag{2}$$

and let

$$G(y_1, \cdots, y_k) = \int_{\substack{a_i' x < y_i \\ i=1,\cdots,k}} \cdots \int dF(x_1, \cdots, x_k). \tag{3}$$

Then, for every continuity interval of $G(y_1, \cdots, y_k)$, we have

$$\Delta_k G_n(\mathbf{a}; \mathbf{b}) \to \Delta_k G(\mathbf{a}; \mathbf{b}), \text{ as } n \to \infty. \tag{4}$$

Here we shall insert one remark. The integral of the right hand side of (3) should, of course, be considered in the Lebesgue-Stieltjes sense. Since $F(x_1, \cdots, x_k)$ was a d.f., there are at most countable hyperplanes which contain points of discontinuities of the function $F(x_1, \cdots, x_k)$ and are parallel to the coordinate planes. If the matrix A is non-singular, the boundaries of the domain of integration of $\Delta_k G(\mathbf{a}; \mathbf{b})$ consist of a finite number of hyperplanes; so we can cover the whole boundaries by a finite number of k-dimensional intervals whose side hyperplanes do not contain any points of discontinuity of $F(x_1, \cdots, x_k)$ and which are arbitrarily small. In other words, the boundary of the domain of integration has the Jordan measure with respect to $F(x_1, \cdots, x_k)$ arbitrarily small; hence the integral $\Delta_k G(\mathbf{a}; \mathbf{b})$ and consequently the integral (3) exist in the Riemann-Stieltjes sense.

If the matrix A is singular, then we may consider the integral $\Delta_k G(\mathbf{a}; \mathbf{b})$ in a sufficiently large but finite k-dimensional interval and take the limit as this interval becomes infinitely large.

To prove (4), it seems to be most convincing to go back to Kolmogrov's original definition of random variable, which was first explicitly stated in his famous booklet [5].

Let the basic probability field under consideration be $(\Omega, \mathfrak{M}, P)$, i.e., let Ω be a certain abstract set consisting of the so-called elementary events, which are denoted by ξ's, and $\mathfrak{M}$ be a Borel field of subsets of Ω containing Ω itself and P be the probability measure defined on the subsets of Ω belonging to $\mathfrak{M}$.

Then the k-dimensional random variable $\mathbf{x}$ is the real-valued and one-valued function defined on Ω such that the inverse image of any k-dimensional Borel set $\mathfrak{A} \in \mathfrak{B}_k$ by $\mathbf{x}$ belongs to $\mathfrak{M}$. This can be written as follows:

$$\{\xi; \mathbf{x}(\xi) \in \mathfrak{A}, \mathfrak{A} \in \mathfrak{B}_k\} \in \mathfrak{M}. \tag{5}$$

It can easily be shown that any Baire function $\mathbf{f}(\mathbf{x})$, i.e., a function defined, for any set of real numbers $a_1, \cdots, a_m$; by the relation

$$\{\mathbf{x}; f_i(\mathbf{x}) < a_i, i = 1, \cdots, m\} \in \mathfrak{B}_k,$$

where $\mathbf{f}(\mathbf{x}) = (f_1(\mathbf{x}), \cdots, f_m(\mathbf{x}))$, is also a m-dimensional random variable (see Kolmogorov, loc. cit. p. 23). So the components $x_1, \cdots, x_k$ of $\mathbf{x}$ as projections of $\mathbf{x}$, are one dimensional random variables.

If we put, for a given number $\epsilon > 0$,

$$Q_n = \{\xi; |\mathbf{z}_n(\xi)| \leq \epsilon\} \quad \text{and} \quad Q_{in} = \{\xi; |\mathbf{z}_{in}(\xi)| \leq \epsilon\}. \qquad i = 1, \cdots, k,$$

then these sets belong to $\mathfrak{M}$ and

$$Q_n \subseteq \bigcap_{i=1}^{k} Q_{in} \equiv Q_n^* \in \mathfrak{M}, \text{ say;}$$

therefore, taking the complements of both sides, we have

$$\bar{Q}_n \supseteq \bar{Q}_n^* \tag{6}$$

From the assumption of the theorem we have $P(\bar{Q}_n) \to 0$, as $n \to \infty$; so it follows that

$$P(\bar{Q}_n^*) \to 0, \qquad \text{as } n \to \infty. \tag{7}$$

Since there are at most countable hyperplanes parallel to the coordinate axes which contain the points of discontinuity of the d.f. $G(\mathbf{y})$, we can assume that, if

$$[\mathbf{a}; \mathbf{b}) = \{\mathbf{x}; a_1 \leq x_1 \leq b_1, \cdots, a_k \leq x_k < b_k\}$$

denotes a continuity interval of $G(\mathbf{y})$, and $\epsilon > 0$ is sufficiently small, then $[\mathbf{a} - \boldsymbol{\varepsilon}; \mathbf{b} + \boldsymbol{\varepsilon})$ and $[\mathbf{a} + \boldsymbol{\varepsilon}; \mathbf{b} - \boldsymbol{\varepsilon})$ are also continuity intervals of $G(\mathbf{y})$.

Now put

$$\begin{aligned} R_n &= \{\xi; \mathbf{a} \leq \mathbf{y}_n(\xi) < \mathbf{b}\}, \\ T_n &= \{\xi; \mathbf{a} - \boldsymbol{\varepsilon} \leq A\mathbf{x}_n(\xi) < \mathbf{b} + \boldsymbol{\varepsilon}\}, \\ U_n &= \{\xi; \mathbf{a} + \boldsymbol{\varepsilon} \leq A\mathbf{x}_n(\xi) < \mathbf{b} - \boldsymbol{\varepsilon}\}. \end{aligned} \tag{8}$$

Then all these sets belong to $\mathfrak{M}$ and

$$U_n \cap Q_n^* \subseteq R_n \cap Q_n^* \subseteq T_n \cap Q_n^*. \tag{9}$$

Indeed, if $\xi \in R_n \cap Q_n^*$, that means that simultaneously

$$\mathbf{a} \leq \mathbf{y}_n(\xi) < \mathbf{b} \text{ and } -\boldsymbol{\varepsilon} < \mathbf{z}_n(\xi) \leq \boldsymbol{\varepsilon};$$

therefore, it follows that

$$\mathbf{a} - \varepsilon \leq A\mathbf{x}_n(\xi) < \mathbf{b} + \varepsilon,$$

and this means that $\xi \in T_n$. Consequently, we have

$$R_n \cap Q_n^* \subseteq T_n \cap Q_n^*.$$

In the same manner, we have

$$U_n \cap Q_n^* \subseteq R_n \cap Q_n^*.$$

Hence we have

$$P(U_n \cap Q_n^*) \leq P(R_n \cap Q_n^*) \leq P(T_n \cap Q_n^*) \tag{10}$$

or

$$P(U_n) - P(U_n \cap \bar{Q}_n^*) \leq P(R_n) - P(R_n \cap \bar{Q}_n^*) \leq P(T_n) - P(T_n \cap \bar{Q}_n^*)$$

or

$$P(U_n) - P(U_r \cap \bar{Q}_n^*) + P(R_n \cap \bar{Q}_n^*) \leq P(R_n) \leq P(T_n) - P(T_n \cap \bar{Q}_n^*) + P(R_n \cap \bar{Q}_n^*). \tag{11}$$

This means that

$$P(U_n) - P(U_n \cap \bar{Q}_n^*) + P(R_n \cap \bar{Q}_n^*) \leqq \Delta_k G_n(\mathbf{a}; \mathbf{b}) \leq P(T_n) - P(T_n \cap \bar{Q}_n^*) + P(R_n \cap \bar{Q}_n^*). \tag{12}$$

We shall define the sets

$$U = \{\mathbf{x}; \mathbf{a} + \varepsilon \leq A\mathbf{x} < \mathbf{b} - \varepsilon\},\ T = \{\mathbf{x}; \mathbf{a} - \varepsilon \leq A\mathbf{x} < \mathbf{b} + \varepsilon\};$$

then we can write

$$\begin{aligned} P(U_n) &= \int_U \cdots \int dF_n(x_1, \cdots, x_k) \quad \text{and} \\ P(T_n) &= \int_T \cdots \int dF_n(x_1, \cdots, x_k) \end{aligned} \tag{13}$$

in the Lebesgue-Stieltjes sense. But in our case the integrals

$$\int_U \cdots \int dF(x_1, \cdots, x_k) \quad \text{and} \quad \int_T \cdots \int dF(x_1, \cdots, x_k) \tag{14}$$

also exist in the Riemann-Stieltjes sense, and, by the assumption of the theorem, $F_n(x_1, \cdots, x_k)$ tends to $F(x_1, \cdots, x_k)$, as $n \to \infty$; so we can see that, as $n \to \infty$,

$$\begin{aligned} P(U_n) &\to \int_U \cdots \int dF(x_1, \cdots, x_k) \equiv \Delta_k G(\mathbf{a} + \varepsilon; \mathbf{b} - \varepsilon) \quad \text{and} \\ P(T_n) &\to \int_T \cdots \int dF(x_1, \cdots, x_k) \equiv \Delta_k G(\mathbf{a} - \varepsilon; \mathbf{b} + \varepsilon). \end{aligned} \tag{15}$$

Hence, as $n \to \infty$, we get from (12) and (15)

$$\Delta_k G(\mathbf{a} + \boldsymbol{\varepsilon}; \mathbf{b} - \boldsymbol{\varepsilon}) \leqq \underline{\lim}_{n\to\infty} \Delta_k G_n(\mathbf{a}; \mathbf{b})$$

(16)

$$\leqq \overline{\lim}_{n\to\infty} \Delta_k G_n(\mathbf{a}; \mathbf{b}) \leqq \Delta_k G(\mathbf{a} - \boldsymbol{\varepsilon}; \mathbf{b} + \boldsymbol{\varepsilon});$$

and, letting $\boldsymbol{\varepsilon} \to 0$, we obtain finally

$$\lim_{n\to\infty} \Delta_k G_n(\mathbf{a}; \mathbf{b}) = \Delta_k G(\mathbf{a}; \mathbf{b}),$$

for any continuity interval $[\mathbf{a}; \mathbf{b})$ of $G(\mathbf{x})$.

If we reflect on the above proof, we have the following generalization at once.

Theorem 3: Suppose that we have for, every index $n = 1, 2, \cdots$,

$$\mathbf{y}_n = \mathbf{f}(\mathbf{x}_n) + \mathbf{z}_n,$$

where $\mathbf{x}_n$ is k-dimensional and $\mathbf{y}_n$; $\mathbf{z}_n$ and $\mathbf{f}(\mathbf{x}_n)$ are k-dimensional random variables. Suppose further that, as $n \to \infty$, the k-dimensional distribution of $\mathbf{x}_n$ tends to a certain limiting distribution, while $\mathbf{z}_n$ converges in probability to zero, and the Baire function $\mathbf{f}(\mathbf{x})$ is such that the set of its discontinuity points has the Jordan measure zero with respect to the limiting distribution function of $\mathbf{x}_n$. Then $\mathbf{y}_n$ has the limiting distribution defined by the transformation

$$\mathbf{y} = \mathbf{f}(\mathbf{x});$$

where $\mathbf{x}$ has the limiting distribution function of $\mathbf{x}_n$.

For example, for the continuous function $\mathbf{f}(\mathbf{x})$, this theorem is true. This theorem is essentially obtained by H. B. Mann and A. Wald [6].

As a further generalization, we have the following.

Theorem 4. Let $F_n(\mathbf{u})$ be the cumulative distribution function of a random vector $\mathbf{u}_n(k \times 1)$ and let $\mathbf{v}_n(h \times 1)$ be a vector-valued function of $\mathbf{u}_n$, i.e.,

$$\mathbf{v}_n(h \times 1) = \mathbf{f}_n(\mathbf{u}_n(k \times 1)),$$

where $\mathbf{f}_n(\mathbf{u}_n)$ is a Baire function. And let $G_n(\mathbf{v})$ be the induced distribution function of $\mathbf{v}_n$. Suppose that $\lim_{n\to\infty} F_n(\mathbf{u}) = F(\mathbf{u})$ at every continuity point of $F(\mathbf{u})$, and suppose that, for every continuity point $\mathbf{u}$ of $\mathbf{f}(\mathbf{u})$, $\lim_{n\to\infty} \mathbf{f}_n(\mathbf{u}) = \mathbf{f}(\mathbf{u})$ uniformly with respect to $\mathbf{u}$. Let $G(\mathbf{v})$ be the d.f. of the random vector $\mathbf{v} = \mathbf{f}(\mathbf{u})$, where $\mathbf{u}$ has the d.f. $F(\mathbf{u})$. If the Jordan measure of the set of discontinuity points of $\mathbf{f}(\mathbf{u})$ with respect to $F(\mathbf{u})$ is zero, then

$$\lim_{n\to\infty} G_n(\mathbf{v}) = G(\mathbf{v})$$

at every continuity point of $G(\mathbf{u})$.

Proof: Let the basic probability field be $(\Omega, \mathfrak{M}, P)$ as before and let the set of k-dimensional space R^k, which consists of the points $\mathbf{u}$ for which the limit function $\mathbf{f}(\mathbf{u})$ has discontinuities, be K, then by the assumptions of the theorem we have

$$\int_K \cdots \int dF(x_1, \cdots, x_k) = 0. \tag{17}$$

where the integral should be considered in the Riemann-Stieltjes sense. Moreover, the integral

$$(18) \qquad G(\mathbf{v}) = \int_{\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}\}} \cdots \int dF(u_1, \cdots, u_k) = \int_{\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}\}\cap\bar{K}} \cdots \int dF(u_1, \cdots, u_k)$$

exists in the Riemann-Stieltjes sense for any real $\mathbf{v}$.

For any continuity point $\mathbf{v}$ of $G(\mathbf{v})$, we get,

$$(19) \qquad G_n(\mathbf{v}) = P(\{\xi; \mathbf{f}_n(\mathbf{u}_n(\xi)) < \mathbf{v}\}) = \int_{\{\mathbf{u};\mathbf{f}_n(\mathbf{u})<\mathbf{v}\}} \cdots \int dF_n(u_1, \cdots, u_k).$$

From the assumptions of the theorem, it follows that, for any $\epsilon' = (\epsilon, \cdots, \epsilon)$, $\epsilon > 0$, we can choose $N(\epsilon)$ such that, for all $n \geq N(\epsilon)$ and for all continuity points of $\mathbf{f}(\mathbf{u})$, we have

$$(20) \qquad \mathbf{f}(\mathbf{u}) - \epsilon < \mathbf{f}_n(\mathbf{u}) < \mathbf{f}(\mathbf{u}) + \epsilon.$$

Hence we have for all $n \geq N(\epsilon)$ and uniformly in $\mathbf{u} \in \bar{K}$,

$$(21) \quad \bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u}) < \mathbf{v} - \epsilon\} \subseteq \bar{K}\cap\{\mathbf{u};\mathbf{f}_n(\mathbf{u}) < \mathbf{v}\} \subseteq \bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u}) < \mathbf{v} + \epsilon\}.$$

On the other hand we have

$$(21) \qquad G_n(\mathbf{v}) = \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}_n(\mathbf{u})<\mathbf{v}\}} \cdots \int dF_n(\mathbf{u}) + \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}_n(\mathbf{u})<\mathbf{v}\}} \cdots \int dF_n(\mathbf{u}).$$

The second term of the right hand side of (21) is dominated by the integral $\int_{\bar{K}} \cdots \int dF_n(\mathbf{u})$ and since the integral (17) exists in the Riemann-Stieltjes sense, we can conclude that

$$(22) \quad 0 \leqq \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}\}} \cdots \int dF_n(\mathbf{u}) < \int_{\bar{K}} \cdots \int dF_n(\mathbf{u}) \to \int_{\bar{K}} \cdots \int dF(\mathbf{u}) = 0, \text{ as } n \to \infty.$$

From (20) it follows that

$$(23) \qquad \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}-\epsilon\}} \cdots \int dF_n(\mathbf{u}) \leqq \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}\}} \cdots \int dF_n(\mathbf{u}) \leqq \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}+\epsilon\}} \cdots \int dF_n(\mathbf{u}).$$

From the relations (21), (22) and (23), we get

$$(24) \qquad \begin{aligned} \lim_{n\to\infty} \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}-\epsilon\}} \cdots \int dF_n(u) &\leq \underline{\lim}_{n\to\infty} G_n(\mathbf{u}) \leq \overline{\lim}_{n\to\infty} G_n(\mathbf{u}) \\ &\leq \lim_{n\to\infty} \int_{\bar{K}\cap\{\mathbf{u};\mathbf{f}(\mathbf{u})<\mathbf{v}+\epsilon\}} \cdots \int dF_n(\mathbf{u}). \end{aligned}$$

Since the integral (18) exists in the Riemann-Stieltjes sense and $F_n(\mathbf{u}) \to F(\mathbf{u})$, as $n \to \infty$, for all continuity points $\mathbf{u}$ of $F(\mathbf{u})$, we can see that

$$G(\mathbf{v} - \epsilon) \leqq \underline{\lim}_{n\to\infty} G_n(\mathbf{v}) \leqq \overline{\lim}_{n\to\infty} G_n(\mathbf{v}) \leqq G(\mathbf{v} + \epsilon); \tag{25}$$

and then, letting $\epsilon \to 0$, we obtain

$$\lim_{n\to\infty} G(\mathbf{v}) = G(\mathbf{v})$$

for all continuity points of $G(\mathbf{v})$.

Theorem 4 is a slight modification of the theorem which T. W. Anderson [1] quoted in his paper as being due to Hermann Rubin.

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STATISTICAL PROBLEMS IN ENERGY LOSS AND IONIZATION PROCESSES[1]

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1. When a beam of monoenergetic charged particles traverses a layer of material, which is thin compared with the range of the particles, the emerging beam contains particles whose energies are distributed statistically. The frequency distribution of the emergent particles as a function of energy has long been known as the "*Landau distribution*" [1]. The traditional method for deriving this distribution is to establish an integro-differential equation for it and then solve by the use of Laplace transforms.

It is instructive to rederive the Landau distribution by a method which throws light on the physical features of the distribution and which, moreover, serves to suggest a simple procedure for obtaining the actual distribution numerically. The fundamental idea is to ask for the conditional probability that

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the total energy loss in the layer of thickness x lies between Δ and $\Delta + d\Delta$ when k inelastic collisions are known to have occurred in the layer. Here we are assuming that passage through the material does not diminish the energy of the particles very much. In fact, we suppose that the layer is thin enough so that the energy loss suffered is always small compared with the initial energy, and that therefore the cross sections are the same for all particles at any stage of their passage through the material. In particular, the probability of an inelastic collision is the same per unit length at every portion of the path within the layer. As a result, the probability that k inelastic collisions occur in the passage of a particle through the material is given by the Poisson distribution:

$$P(k) = \frac{\bar{k}^k}{k!} e^{-\bar{k}} \tag{1}$$

where $\bar{k}$ is the mean number of inelastic collisions in the layer.

The conditional probability with which we want to fold this distribution can be constructed from a knowledge of the elementary collision cross section as a function of the energy loss incurred in an individual encounter. Let $w(\eta)\, d\eta$ be the probability that a particle which possesses the given incident energy E_0 loses an energy between η and $\eta + d\eta$ in an atomic collision within a unit length of traversed matter. Here we assume that the unit of length has been chosen short enough such that the total probability W for an inelastic collision is still small compared with unity:

$$W = \int_0^\infty w(\eta) d\eta \ll 1 \tag{2}$$

(To be precise, one should extend the integration only to the total energy E_0 possessed by the particle, instead of infinity, but the difference is negligible, because in practice $w(\eta)$ is always a rapidly decreasing function of η.) Of course, w and W depend on E_0, but we shall not indicate this fact explicitly. Condition (2) assures that the occurrence of more than one collision per unit length can be neglected. We note that

$$w(\eta) = n\sigma(E_0, \eta) \tag{3}$$

where n is the number of atoms per unit volume of the material, and $\sigma(E_0, \eta)$ is the atomic cross section for inelastic collisions with energy loss η per unit energy interval. Evidently we also have the connection:

$$\bar{k} = Wx \tag{4}$$

The probability that an inelastic collision, *known to have occurred* within a given unit length, will result in an energy loss between η and $\eta + d\eta$ is given as:

$$\frac{w(\eta)\, d\eta}{W} \tag{5}$$

It now takes no effort to write down immediately the compound probability that an energy between Δ and $\Delta + d\Delta$ was lost in the thickness x; we denote this probability by $f(x, \Delta)\, d\Delta$, and have:

$$f(x, \Delta)\, d\Delta = \sum_{k=0}^{\infty} P(k) \int_0^{\Delta} \frac{w(\eta_1)\, d\eta_1}{W} \int_0^{\Delta-\eta_1} \frac{w(\eta_2)\, d\eta_2}{W} \cdots \int_0^{\Delta-\eta_1-\eta_2-\cdots-\eta_{k-2}} \frac{w(\eta_{k-1})\, d\eta_{k-1}}{W} \frac{w(\Delta - \eta_1 - \eta_2 - \cdots - \eta_{k-1})\, d\Delta}{W} \tag{6}$$

If negative energy losses (i.e. inelastic collisions of the second kind) do not occur, $w(\eta) = 0$ for $\eta < 0$, and all upper limits of integration may be taken to be infinity with impunity. At the same time, we substitute equation (1) into (6), and we put the latter equation into a more symmetric form by use of the identity:

$$w(\Delta - \eta_1 - \eta_2 - \cdots - \eta_{k-1}) = \int_0^{\infty} w(\eta_k)\delta(\Delta - \eta_1 - \eta_2 - \cdots - \eta_k)\, d\eta_k$$

The result of all these transformations is:

$$f(x, \Delta) = \sum_{k=0}^{\infty} \frac{x^k}{k!} e^{-Wx} \int_0^{\infty} \int_0^{\infty} \cdots \int_0^{\infty} w(\eta_1)w(\eta_2)\cdots w(\eta_k) \times \delta(\Delta - \eta_1 - \eta_2 \cdots - \eta_k)\, d\eta_1\, d\eta_2 \cdots d\eta_k \tag{7}$$

It is apparent in equation (7) that the delta function guarantees that the total energy loss adds up to the required amount Δ.

If we substitute the familiar representation for the delta function:

$$\delta(x) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} e^{isx}\, ds$$

into equation (7) we obtain:

$$f(x, \Delta) = \frac{1}{2\pi} \sum_{k=0}^{\infty} \frac{x^k}{k!} e^{-Wx} \int_{-\infty}^{+\infty} e^{is\Delta} \left[\int_0^{\infty} w(\eta) e^{-is\eta}\, d\eta \right]^k ds$$

which can be summed to give:

$$f(x, \Delta) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} \exp[is\Delta - xF(s)]\, ds \tag{8}$$

where:

$$F(s) = \int_0^{\infty} w(\eta)[1 - e^{-is\eta}]\, d\eta = W - \int_0^{\infty} w(\eta) e^{-is\eta}\, d\eta \tag{9}$$

Equations (8) and (9) are in complete agreement with Landau's formulas.

Our derivation, leading in steps to the expression of equation (6), suggests a simple Monte Carlo method for obtaining $f(x, \Delta)$. For instance, one might use a Geiger counter which is set to deliver an average of $\bar{k} = Wx$ pulses in a fixed time interval. Each pulse triggers a roulette wheel whose circumference is divided according to the cross section for various energy losses η. An automatic adding device receives the k values of η and produces the total energy loss as their sum.

Landau and others [2–5] have evaluated the distribution $f(x, \Delta)$ directly by numerical integration in addition to some approximate methods. The atomic cross sections which enter these calculations are those familiar from Bethe's pioneering work on atomic collisions [6].

2. Once the Landau distribution has been calculated, it becomes desirable to compare it with experimental results. Such experimental verification is direct and straightforward if the energy loss Δ of each particle can be measured by determining its energy before entering the material and after leaving it. In many experiments this cannot be done with sufficient accuracy, and one seeks instead to measure the energy loss Δ directly by determining how much energy has been absorbed by the material from the particle. Typically, a fast μ meson traverses a certain thickness of a gas, and its energy diminishes by a few kev from its original value of several hundred Mev. Unfortunately, a direct energy measurement is again impossible, and one attempts instead to count the total number of ion pairs produced by the ionizing primary and the resulting secondary radiation. It is then commonly *assumed* that the energy loss of the primary particle is *strictly proportional* to the total number of ion pairs produced.

Moyal [5], in an effort to avoid this assumption, has derived the distribution of the number of ion pairs directly. The procedure is similar to, although somewhat more complicated than, Landau's derivation of the distribution of energy losses. We shall refer to the resulting distribution as the "modified Landau distribution." Moyal's calculation requires the knowledge of the probability q_k that a primary ionizing collision, known to have occurred, results in the creation of k ion pairs. Moyal conjectured the behavior of the quantities q_k . It would be desirable to calculate them independently.

Similarly, it seemed worthwhile to calculate the average energy expended per ion pair produced and to examine to what extent this quantity is as independent of the primary energy and the nature of the incident particle as is usually assumed [7]. Furthermore, one would like to calculate the variance of the number of ion pairs, since reliable conclusions about the energy loss of the primary can be drawn from the observed number of ion pairs only if, in addition to the mean number being proportional to the energy loss, this distribution has a *narrow* peak.

Fano [8, 9] has made some interesting semi-empirical calculations of means and variances, but the actual distribution has not been calculated. We shall outline the formal developments of such a calculation in the following section.

3. In the description of the ionization process it will be well to distinguish the *primary* ionizing collisions, in which the fast incident particle collides with an atom of the gas and ejects an electron, from all collisions which follow such an event. We refer to the latter as *secondary* collisions. They are between comparatively slow electrons and atoms. A cascade of secondary ionizing collisions develops if the ejected electron has sufficient energy. The central quantity to be calculated is *the probability $P_n(E)$ that an electron of energy E*, which has been released in the gas at some stage of this cascade, *will lead to the production of a*

total of n ion pairs before it and all the other electrons, which it liberates directly or indirectly, come to rest. In other words, we shall assume that wall effects are absent and that every electron comes ultimately to rest in the gas by expending its energy in inelastic (i.e. ionizing and exciting) collisions with the atoms of the gas.

Evidently we have

$$\sum_{n=0}^{\infty} P_n(E) = 1. \tag{10}$$

We now make the following definitions:

$p_e(E, \epsilon_j)$: Probability that an inelastic collision of an electron with energy E leads to an energy loss ϵ_j by atomic excitation.

$p_i(E, \epsilon)$: Probability that an inelastic collision of an electron with energy E leads to an energy loss $\epsilon + I$ by ionization, I being the ionization energy, and ϵ the kinetic energy of the ejected electron.

If the excited states of the atom have energies $\epsilon_1, \epsilon_2, \cdots$ above the ground state, one infers readily the fundamental equation [10]:

$$P_n(E) = \sum_{j=1}^{\infty} p_e(E, \epsilon_j) P_n(E - \epsilon_j) + \int_0^{E-I} p_i(E, \epsilon) \sum_{k=0}^{n-1} P_k(\epsilon) P_{n-k-1}(E - \epsilon - I)\, d\epsilon \tag{11}$$

valid for $n = 0, 1, \cdots$ and for $E > \epsilon_1$. This set of equations can be solved in successive steps starting from $P_0(E)$, since the right hand side involves the knowledge of P_n only for values of the argument less than E. The equations are subject to simple physical boundary conditions. Thus

$$P_0(E) = 1 \qquad \text{for } E < I \tag{12}$$

and generally:

$$P_n(E) = 0 \qquad \text{for } E < nI \tag{13}$$

For $n = 0$ we obtain:

$$P_0(E) = \sum_{j=1}^{\infty} p_e(E, \epsilon_j) P_0(E - \epsilon_j) \tag{14}$$

Substituting (12) in this equation, if $E - \epsilon_j < I$, we find

$$P_0(E) = \sum_{j=1}^{\infty} p_e(E, \epsilon_j) \quad \text{for} \quad I < E < I + \epsilon_1. \tag{15}$$

This equation merely states the obvious fact that, if E is in the given range, any excitation process deprives the electron of enough energy to prevent subsequent ionization. If we now substitute (15) on the right hand side of (14), we can compute $P_0(E)$ in the range from $I + \epsilon_1$ to $I + 2\epsilon_1$, and so on in steps of ϵ_1. Having thus obtained $P_0(E)$ for all relevant energies, one can now go back

to equation (11) and compute $P_1(E)$ again step by step in analogous manner. These calculations can be simplified if one replaces the infinitely many excited states by a single equivalent excited state. This can be done without great loss of accuracy, particularly in the case of helium, in which all excited states lie very near the ionization limit. One determines the mean excitation energy $\bar{\epsilon}$ by expanding $P_n(E - \epsilon_j)$ in a Taylor series in the neighborhood of $\bar{\epsilon}$, and by requiring that the first-order corrections in equation (11) vanish:

$$\sum_{j=1}^{\infty} p_\epsilon(E, \epsilon_j)(\bar{\epsilon} - \epsilon_j) = 0 \tag{16}$$

Hence $\bar{\epsilon}$ is the center of gravity of the distribution of excitation energy losses. This procedure is successful because $\bar{\epsilon}$ so defined is only slowly dependent on E.

Returning to the probabilities $P_n(E)$, it is convenient to define a generating function $F(z, E)$ by the relation:

$$F(z, E) = \sum_{n=0}^{\infty} z^n P_n(E). \tag{17}$$

From (11) one infers for the generating function the non-linear integral equation:

$$F(z, E) = \sum_{j=1}^{\infty} p_\epsilon(E, \epsilon_j) F(z, E - \epsilon_j) + z \int_0^{E-I} p_i(E, \epsilon) F(z, \epsilon) F(z, E - \epsilon - I)\, d\epsilon \tag{18}$$

The initial conditions are

$$F(1, E) = 1 \qquad \text{for all } E$$

and

$$F(z, E) = 1 \qquad \text{for } E < I.$$

The mean value and the variance of the distribution of secondary ion pairs can be obtained easily from these equations. Thus:

$$J(E) = \langle n \rangle = \sum_{n=0}^{\infty} n P_n(E) = \left(\frac{\partial F}{\partial z}\right)_{z=1}$$

$$= \sum_{j=1}^{\infty} p_\epsilon(E, \epsilon_j) J(E - \epsilon_j) + \int_0^{E-I} p_i(E, \epsilon)\,[J(\epsilon) + J(E - \epsilon - I) + 1]\, d\epsilon, \tag{19}$$

a linear integral equation.[2] A similar, but more complicated, relation can be derived for the variance.

[2] G. A. Erskine, Proc. Roy. Soc. **A 224:** 362 (1954). See also R. H. Fowler, Proc. Camb. Phil. Soc. **21:** 531 (1923). G. W. Griffing (private communication) has also considered this equation. For details on the application to helium see W. F. Miller, Ph.D. thesis, Purdue University, 1956.

TABLE I

Excitation cross sections $\sigma_n(E)$ and $\sigma_{exc}(E)$

E	$\sigma_2(E)$	$\sigma_3(E)$	$\sigma_4(E)$	$\sigma_5(E)$	$\sigma_{exc}(E)$
1	1.2868	0.1787	0.0468	0.0199	1.5629
2	1.4933	0.2790	0.1017	0.0488	1.9985
3	1.3056	0.2373	0.0893	0.0429	1.7417
4	1.1424	0.2121	0.0775	0.0372	1.5269
5	1.0146	0.1874	0.0683	0.0328	1.3539
6	0.9134	0.1678	0.0609	0.0293	1.2168
7	0.8359	0.1523	0.0554	0.0265	1.1112
8	0.7659	0.1395	0.0507	0.0243	1.0190

4. As an example of the theory of section 3, we have calculated the probabilities that an electron of energy E (between 0 and 12 Rydbergs) produces directly or indirectly 0, 1, 2 or 3 ion pairs when released in *atomic hydrogen*. This example was chosen because theoretical cross sections are available and are expected to be fairly accurate, even for molecular hydrogen, except at the lowest energies.[3] It would be preferable to employ atomic cross sections throughout which have been shown experimentally to be reliable, but unfortunately not many measurements have been carried out. In particular, the spectrum of secondary electrons from electron impact is an important input datum about which little is known experimentally. The numerical results of the calculations must be judged in the light of this situation. Indirectly they provide a certain test of the assumptions made about basic cross sections.

All cross sections were obtained by evaluating the formulas which Bethe has derived.[3] We quote some of the numerical results, since they might be useful in other calculations of a similar nature. Table I lists the cross section $\sigma_n(E)$ for excitations to an excited level of principal quantum number n, as a function of the incident energy E, as well as the *total excitation cross section*. The levels $n = 6$ to ∞ never contribute more than 5% to the total cross section. Table II lists a few values of cross sections for ionization, ϵ being the kinetic energy of the ejected electron. All energies are measured in Rydbergs (= 13.6 eV), and all cross sections in units of πa^2, where a is the Bohr radius of hydrogen.

For higher energies simple formulas, similar to those given by Bethe, were used to approximate the cross sections.[4]

From the cross sections one infers the total excitation probability:

$$p_e(E) = \sum_{j=1}^{\infty} p_e(E_1 \epsilon_j) = \frac{\sigma_{exc}(E)}{\sigma_{ion}(E) + \sigma_{exc}(E)} \tag{20}$$

[3] H. A. Bethe, Handbuch der Physik, vol. 24(1), Berlin, 1933, p. 273. See also reference 9. A recent exhaustive summary of theoretical and experimental work on collisions of electrons is found in the article of H. S. W. Massey: "Excitation and Ionization of Atoms by Electron Impact", Encyclopedia of Physics, vol. 36(II), Berlin, 1956, p. 307.

[4] For details see J. R. Herring, M.S. thesis, University of North Carolina, 1956. Excitation cross sections have also been calculated by R. McCarroll, Proc. Phys. Soc. (London) **A 70:** 460 (1957). Most of his numerical results are in good agreement with ours as presented in Table I.

TABLE II

Ionization cross section $\sigma_{ion}(E, \epsilon)$ *and* $\sigma_{ion}(E)$

E	$\sigma_{ion}(E, 0)$	$\sigma_{ion}(E, 0.3)$	$\sigma_{ion}(E, 0.6)$	$\sigma_{ion}(E, 1.0)$	$\sigma_{ion}(E, 1.5)$	$\sigma_{ion}(E, 2.0)$	$\sigma_{ion}(E)$
1.5	2.673	1.013					
2.0	2.724	1.297	0.670				1.007
3.0	2.417	1.199	0.699	0.384	0.194		1.204
4.0	2.102	1.038	0.609	0.376	0.196	0.118	1.200

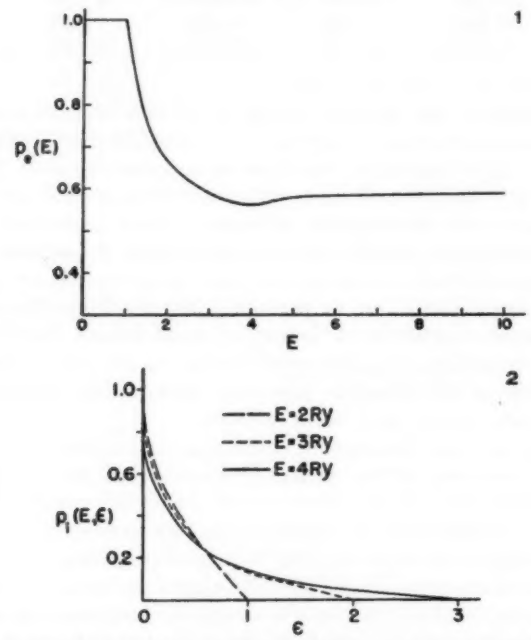


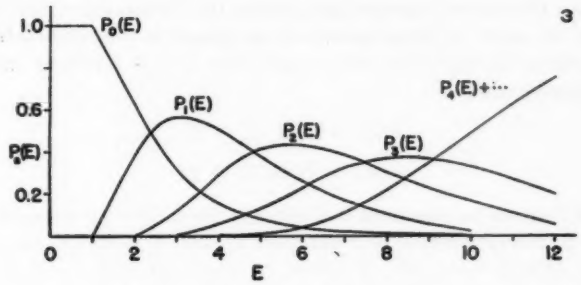
FIG. 1. Total excitation probability $p_e(E)$ per inelastic event as a function of the energy, E, of the incident electron. The abscissa is in Rydberg units. FIG. 2. Ionization probability $p_i(E, \epsilon)$ per inelastic event, as a function of the incident energy, E, and the kinetic energy, ϵ, of the secondary. Energies are measured in Rydbergs.

This quantity is plotted in Figure 1. Similarly, we calculate the ionization probabilities $p_i(E, \epsilon)$ from

$$p_i(E, \epsilon) = \frac{\sigma_{ion}(E, \epsilon)}{\sigma_{ion}(E) + \sigma_{exc}(E)} \tag{21}$$

and this quantity is shown in Figure 2 for a few values of E.

Using these probabilities in equation (11) we then computed $P_n(E)$ for atomic hydrogen. The results are shown in Figure 3. The curves for $n = 0$ to $n = 3$ were obtained by numerical calculation. When these computations are performed



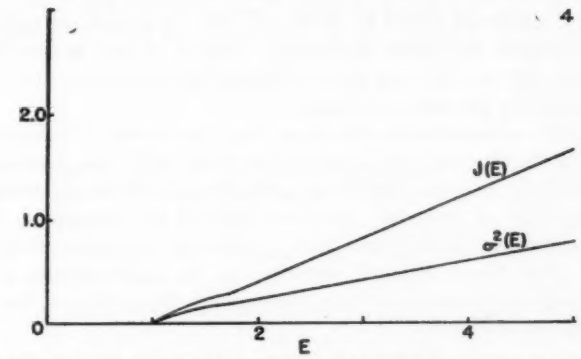


FIG. 3. $P_n(E)$ versus E (in Rydbergs). For definitions see the text. FIG. 4. Mean $J(E)$ and variance $\sigma^2(E)$ of the number of ion pairs versus initial energy (in Rydbergs).

with a desk calculator, appreciable rounding-off errors make the results increasingly uncertain for higher values of n and E. Since the present calculation was intended mainly as the study of a reasonably realistic model, no particular effort was made to keep such errors to a minimum. Generally, we estimate that the results shown in Figure 3 are accurate to within about 5 per cent provided that the atomic probabilities of Figures 1 and 2 are regarded as free from error. It might be observed that, if very trustworthy cross sections for the inelastic collisions were available, equation (11) should be solved on an electronic digital computer.

On Figure 3 the curve which rises monotonically with energy represents the sum of all $P_n(E)$ from $n = 4$ to ∞.

5. The mean number, $J(E)$, of ion pairs produced, as a function of initial energy E, was computed directly from the distribution for $E < 5$ Rydberg. Similarly $\sigma^2(E)$ was obtained by using the formula:

$$\sigma^2(E) = \langle (n - J)^2 \rangle = \sum_{n=0}^{\infty} [n - J(E)]^2 P_n(E) \tag{22}$$

The results are given in Figure 4.

Apart from the curved portion just above the threshold, which is of minor significance, the most striking feature of this graph is the remarkable linearity of both functions at relatively low energies. For $E < 5$ Rydberg one can write to good approximation:

$$J(E) = 0.42\ (E - I) \tag{23}$$

$$\sigma^2(E) = 0.18\ (E - I) \tag{24}$$

where E and I are to be measured in Rydbergs. From (23) we see that the energy which leads to the production of J ion pairs is $(32J + 13.6)$ eV. For high initial energies the additive term can, of course, be neglected, and one may then speak of "the average energy expended per ion pair." However, the numerical value of this quantity, which we found to be 32 eV, may be greater at higher energies because the relative excitation probability (Figure 1) rises gradually. The approximate linearity of $J(E)$ can also be inferred from equation (19) as has been done by Erskine for the case of helium.[2]

So far, all the calculations in this paper have pertained to inelastic collisions of electrons in the electron volt energy region. Our results are, however, of much wider applicability, because when a fast primary particle passes through the gas, the great majority of secondary electrons released has energies in this region. Detailed calculations of the mean total number of ion pairs produced by the passage of a fast heavy charged particle (e.g. an alpha-particle of one Mev energy) through hydrogen have been made using the results of this paper and will be presented elsewhere.[5]

Here we shall merely discuss one special application, that of calculating the quantities q_k which enter Moyal's derivation [5] of the modified Landau distribution of the number of ion pairs, as discussed in sections 1 and 2. $q_k(E)$ is the probability that an ionizing collision which the primary particle has suffered will lead to the production of k ion pairs in the course of the complete degradation of energy. Consequently $q_k(E)$ is given by:

$$q_k(E) = \int_0^\infty \frac{\sigma^a_{\text{ion}}(E, \epsilon)}{\sigma^a_{\text{ion}}(E)} P_{k-1}(\epsilon)\, d\epsilon \tag{25}$$

where $\sigma^a_{\text{ion}}(E, \epsilon)$ is the cross section for ionization with ejection of an electron of kinetic energy ϵ by a heavy (α-) particle of energy E. $\sigma^a_{\text{ion}}(E)$ is the corresponding total ionization cross section. The upper limit of the integration can be taken to be infinite since the contributions to the integral become vanishingly small for large ϵ.

As an example, $q_k(E)$ was evaluated for α-particles of 1 Mev energy, and the results are listed in Table III. In order to test Moyal's conjecture that q_k can be represented by the formula:

$$q_k = \frac{a}{k^2 + 1} \tag{26}$$

[5] The experimental results are summarized in reference 10.

TABLE III

q_k for 1 Mev α-particles

k	q_k	q_k/q_4	$17/(k^2+1)$	$16/k^2$
1	0.787	34.22	8.50	16.00
2	0.097	4.22	3.40	4.00
3	0.041	1.78	1.70	1.78
4	0.023	1.00	1.00	1.00

we have tabulated the ratios q_k/q_4 and, for comparison, the ratios $(4^2+1)/(k^2+1)$, and also $4^2/k^2$. The results bear out Moyal's contention that q_k is inversely proportional to k^2 for higher values of k. In fact, excellent agreement with the values of q_2 and q_3 can be obtained if instead of (26) one adopts the representation

$$q_k = \frac{a'}{k^2} \qquad (k = 2, 3, \cdots) \quad (27)$$

and adjusts the constant a' to give exact agreement with q_4. Moyal prefers equation (26) because it seemed applicable even for $k = 1$. Indeed, if *classical* cross sections are used for the collisions, q_1/q_2 can be shown to have very nearly the value 2.5, which agrees with the prediction of (26). However, our results show that q_1/q_2 is as large as 8 when quantum mechanical cross sections are employed. While we agree with Moyal's *ratios* of the q_k for higher values of k, we conclude that the actual numerical values, which he determined by requiring

$$\sum_{k=1}^{\infty} \frac{a}{k^2+1} = 1,$$

are considerably in error. For the present example of 1 Mev alpha-particles in hydrogen we might adopt the values:

$$q_1 = 0.787 \qquad q_k = \frac{0.368}{k^2} \qquad \text{for } k = 2, 3 \cdots \quad (28)$$

Moyal's assumption that these values are not sensitively dependent on the primary energy E is a reasonable one, even when quantum mechanical cross sections are used. It remains to be seen whether the modification of Moyal's calculations, proposed here, can lead to agreement between the theoretically predicted Landau type distribution of the number of ion pairs and the experimental observations. The latter seem to indicate, at least under certain conditions, a greater width of the distribution than theoretically expected.[6]

6. Finally, we exhibit a simple *approximate formula* for $P_n(E)$ and compare it with the distribution which was obtained in section 4 by numerical evaluation. For a fixed available energy to be dissipated in the gas, one expects that the

[6] For a summary of older work see D. West, Progress in Nuclear Physics, vol. III, London, 1953, p. 18. Recent measurements were made by T. E. Gilmer and E. D. Palmatier (to be published).

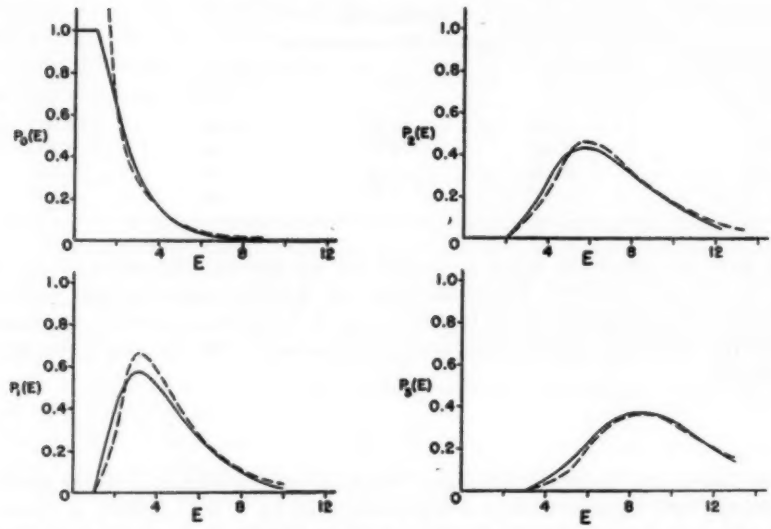


FIG. 5. The solid curves represent $P_n(E)$ as evaluated numerically. The dashed curves are the approximation of equation (29). E is measured in Rydbergs.

number, n, of ion pairs will be distributed approximately according to a normal distribution around its mean, $J(E)$, with a variance $\sigma^2(E)$. If we assume that the mean and the variance are both proportional to $E - I$, as indicated by (23) and (24), we may suppose that $P_n(E)$ is given by the formula:

$$P_n(E) = \frac{1}{\sqrt{2\pi\alpha(E - I)}} \exp \frac{[n - \lambda(E - I)]^2}{2\alpha(E - I)} \tag{29}$$

Here λ and α are defined by:

$$J(E) = \lambda(E - I) \tag{30}$$

$$\sigma^2(E) = \alpha(E - I) \tag{31}$$

The values of λ and α could be taken from equations (23) and (24). However, we have instead determined these two parameters in (29) by matching the maximum of $P_3(E)$ as calculated from (29) with the peak of the $P_3(E)$ curve in Figure 3. This requirement gave:

$$\lambda = 0.377\text{Ry}^{-1}, \qquad \alpha = 0.149\text{Ry}^{-1}$$

if all energies in (30) and (31) are measured in Rydbergs. Figures 5a–d show the comparison of our normal approximation with the "exact" curves. The detailed skew features of the latter are well reproduced even for small values of n. The simple formula (29) can be used for purposes of orientation, when one wishes to calculate various physical quantities, such as q_k, which depend on the distribution of the ion pairs produced by secondary electrons.

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STABILITY OF METAL-TRIETHYLENETETRAMINE COMPLEXES

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Certain metal ions (such as cobalt, nickel, copper, zinc, cadmium, and mercury) form more stable bonds with nitrogen than with oxygen (9). Such metal ions in ammoniacal solution form stable ammine complexes. In contrast, in ammoniacal solution, certain other metal ions (such as alkaline and rare earths, aluminum, bismuth, and scandium) either do not react or form hydrous oxide precipitates rather than stable ammine complexes. Thus, chelons such as polyamines, which have only nitrogens as the coordinating atoms, would be expected to complex with a restricted set of metal ions.

Triethylenetetramine (abbreviated 'trien'), which has the following structure

$$H_2N—CH_2CH_2NHCH_2CH_2NHCH_2CH_2—NH_2$$

seemed to be an interesting example of such a polyamine and was selected for study.

DETERMINATION OF METAL-TRIEN STABILITY CONSTANTS.—Among the various methods (3) employed for the determination of equilibrium constants for chelate formation, the original (1) and modified (8) Bjerrum method and the polarographic (2) method have been most frequently used. The original Bjerrum

method is applicable only to chelates of low or intermediate stability. It requires considerable time for the experiments and tedious calculations are involved. The Bjerrum method was modified by Schwarzenbach and Freitag (8) for the determination of stability constants for chelates of high stability but the work required in determining the stability constants became even greater.

A potentiometric method devised by the authors (7) affords a *rapid* and *simple* determination of stability constants of 1:1 metal chelates (where 1 mole of chelating agent reacts with 1 mole of metal ion) under equilibrium conditions.

Principle.—A combination of the Nernst equation for a mercury electrode

$$E = E^{\circ}_{Hg} + 0.0296 \log [Hg^{++}]$$

with the equations for the stability constants of the 1:1 mercury trien complex

$$K_{HgZ} = \frac{[HgZ^{++}]}{[Hg^{++}][Z]}$$

and a 1:1 metal (Me^{++}) trien complex

$$K_{MeZ} = \frac{[Me^{++}]}{[Me^{++}][Z]}$$

gives at 25°

$$E_{Hg} = E^{0}_{Hg} + 0.0296 \log \frac{[Me^{++}][HgZ^{++}]}{[MeZ^{++}]K_{HgZ}} + 0.0296 \log K_{MeZ} \qquad (1)$$

From equation 1, the potential of the mercury electrode is seen to depend *linearly* on log K of the particular metal chelate involved, provided the concentrations of the mercury chelate, the metal ion, and the metal chelate are kept constant. The log K of the metal chelate complex can be calculated readily from experimental data provided the value of the stability constant for the mercury complex, K_{HgZ}, is known.

The latter can also be determined experimentally from potential measurements with the mercury electrode. The potential of a mercury electrode in a solution containing free trien and mercuric trien chelate is given at 25° by

$$E = E^{0}_{Hg} + 0.0296 \log \frac{[HgZ^{++}]\phi}{[Z]_t K_{HgZ}} \qquad (2)$$

where $[Z]_t$ corresponds to the stoichiometric concentration of trien,

$$\phi = 1 + \frac{[H^+]}{K_4} + \frac{[H^+]^2}{K_4 \cdot K_3} + \frac{[H^+]^3}{K_4 \cdot K_3 \cdot K_2} + \frac{[H^+]^4}{K_4 \cdot K_3 \cdot K_2 \cdot K_1}$$

and K_4, K_3, K_2, and K_1 are the acidity constants of trien.

With a knowledge of ϕ, K_{HgZ} can then be calculated from the measured potential-pH function and the concentrations involved. Curve I in Fig. 1 shows this experimentally determined potential as a function of pH for a solution which contains equal amounts of free chelating agent (trien) and the corresponding mercuric-trien complex.

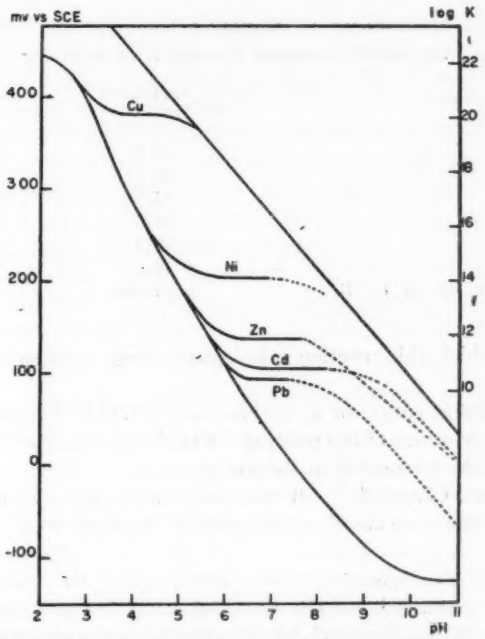

FIG. 1. Potential-pH diagram for determination of trien stability constants: 25° and ionic strength of 0.1 (KNO_3): I, 0.001 M HgZ^{++} + 0.001 Z: II, calculated (W. M. Latimer, "Oxidation Potentials," Prentice-Hall, Inc., New York, N. Y., 1952, p. 179) potential for $Hg + 2OH^- \rightarrow HgO + H_2O + 2e^-$; other lines, 0.001 M HgZ^{++} + 0.001 M MeZ^{++} + 0.001 M Me^{++}; Note: line I is obtained in the case of Mg, Ca, Sr, Ba, La, Al, and Bi.

Alternatively, K_{HgZ} can be determined from the potential of curve I at high pH values (>11) where trien exists only in the free base form and $\phi = 1$. In this manner the stability constant of HgZ can be obtained without a knowledge of the acidity constants of the trien.

With the help of this constant, K_{HgZ}, a log K scale was calculated from equation 1 and plotted at the right hand side of Fig. 1. Next, a potential-pH diagram was constructed from experimental data for each of several metal ions in such a way that, for definite concentrations of the chemicals involved in equation 1, the log K value for the metal chelate could then be read directly from the measured potential of the mercury electrode, in its pH-independent region (Fig. 1). A difference in log K of one unit corresponds to a difference of 29.6 mv. in potential.

Because curve I is obtained in the presence of Mg, Ca, Sr, Ba, La, Al, and Bi, no stable trien complex with these metals exists. The dotted lines, in the cases of metals which do complex, represent the hydrolysis of these metal ions.

This potentiometric method for determining metal stability constants also can be considered as an exchange reaction

$$HgZ^{++} + Me^{+\nu} \rightleftarrows MeZ^{+\nu} + Hg^{++} \qquad (3)$$

TABLE I

Log stability constants for metal-trien complexes

Metal	Log K (Hg electrode) (25.0°C, μ = 0.1)	Log K (4) (20°C, μ = 0.1)
Hg	25.0	25.3 (μ = 0.5)
Cu	20.1	20.4
Ni	14.1	14.0
Zn	11.9	12.1
Cd	10.8	10.7
Pb	10.4	—
Mg, Ca, Sr, Ba, Al, La, Bi	negligible	—

the extent to which this reaction takes place being measured by the mercury electrode.

Results.—Stability constants of various metal-EDTA chelate complexes have been measured by means of the method outlined above and are listed in Table I, together with values reported in the literature.

EXPERIMENTAL. *Chemicals.*—All chemicals except trien were AR grade. The metal ion solutions were standardized against standard trien and EDTA solutions.

Purification of Triethylenetetramine.—Recrystallize the commercial product (Union Carbide Chemicals Co.) as the disulfate in the following manner (or as the tetranitrate in a similar way). The tetranitrate form is employed when metal ions, e.g. barium, are present which would precipitate as sulfate. Mix together 60 ml. of the amine, 400 ml. of water, and 100 ml. of 95 per cent ethanol. Add slowly with stirring 276 ml. of 1:5 sulfuric acid (i.e., 46 ml. of concentrated acid and 230 ml. of water). The pH should be less than 2; if not, add more sulfuric acid. Cool the mixture in an ice bath. Filter the crystals; wash twice with 80 ml. of 1:1 ethanol-water. Redissolve the crystals in 550 ml. of hot water and filter the hot solution. Add to the filtrate slowly and with stirring 185 ml. of hot 95 per cent ethanol. Filter the crystals; wash three times with 80 ml. of 1:1 ethanol-water, dry at 80–100°C *in vacuo* (water pump). The yield of disulfate is 55 to 65 per cent. Further recrystallization is desirable if the molarity of the standard chelon solution is to be 0.01 or smaller. The excess of sulfuric acid must be washed out; otherwise the dried product will decompose with time.

Determination of Potential-pH Diagrams.—The cell consisted of a 50-ml. beaker surrounded by a condenser-type jacket connected in turn to a constant temperature bath maintained at 25.0 ± 0.1°. A glass electrode (Beckman no. 1199-30), a J-tube mercury electrode (5, 6) and a saturated calomel electrode with an agar bridge were used. About 30 ml. of solution was taken for a measurement and stirred continuously by means of a magnetic stirrer. The pH was varied by dropwise addition of sodium hydroxide or perchloric acid. The pH was measured with a Leeds and Northrup pH meter (type 7664) and the potential by means of a Leeds and Northrup student potentiometer. Equilibrium within ±1 mv. generally was reached in about a minute. Deaeration of the solution was

necessary in order to obtain accurate potentials in the more negative potential regions where the presence of oxygen interferes ("mixed potentials"). The cell therefore was closed with a cork stopper provided with the necessary holes and nitrogen was bubbled through the solution.

EXTENSIONS AND FURTHER APPLICATIONS OF THE SYSTEM. *Mercury as a pM Indicator Electrode.*—From equation 1, it may be seen that the potential of the electrode may also bear a *linear* relationship with log [Me] and consequently the electrode may serve as a pM indicator electrode (analogous to a pH indicator electrode) in the presence of fixed concentrations of the metal chelate and mercuric chelate. This feature is particularly significant in situations where suitable electrodes of the first class, i.e., a metal electrode in contact with a solution containing the metal ion, are not applicable—such as with the alkaline earth metals. Thus, for example, the electrode system

$$Hg/Hg\text{-}EDTA^{-2}, Ca\text{-}EDTA^{-2}, Ca^{++}$$

would be, within certain pH limits, a pCa electrode in the presence of fixed added concentrations of mercuric-EDTA and calcium-EDTA complexes.

Such a pM indicator electrode could be used to study the interaction of a metal ion (e.g., Ca^{++}) with many types of complexing agents provided the complexing agent does not react with the metal ion more strongly than the reference chelate (e.g., EDTA).

With trien as the chelating agent a more *selective* pM indicator electrode is obtained since metal ions such as Mg, Ca, Sr, Ba, La (and possibly other rare earths), Al, and Bi would not interfere.

This principle of the pM indicator electrode can and has been applied to the potentiometric detection of end-points in chelation titrations involving a variety of metals, chelating agents and conditions.

Metal-Ion Buffer Solutions.—Consideration of Fig. 1 points out another possible application. The potential is, of course, a measure of the Hg^{++} activity and Fig. 1 illustrates that the activity of mercuric ions can be adjusted to almost any desired level by addition of a certain metal ion and its corresponding trien complex to a solution of mercuric-trien complex. The particular metal ion selected allows the pHg level to be adjusted close to the desired value, e.g., Pb^{++} for high pHg levels, Ni^{++} for intermediate values, Cu^{++} for low levels. The relative concentrations of these ingredients may be varied to allow fine adjustments in the pHg level. Addition or removal of mercuric ions in small amounts will result in only small changes in the pHg level, hence a *buffered* solution in respect to the mercuric ion. *An important feature of this technique is that the pHg level remains virtually constant over a reasonable pH range.*

Obviously the method is not restricted to pHg levels but can also be employed to obtain *pM levels of almost any metal ion that forms 1:1 complexes.*

The selectivity of trien allows control of pM values for Hg, Ni, Zn, Cd, and Pb with no influence from the metal ions Mg, Ca, Sr, Ba, La (and possibly other rare earths), Al and Bi.

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STANDARD POTENTIALS IN AQUEOUS HYDROCHLORIC ACID SOLUTIONS CONTAINING ORGANIC SUBSTANCES. ELECTROMOTIVE FORCE STUDIES WITH D-SORBITOL

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The work reported on in this paper is a continuation of the studies that have been carried on in this laboratory with cells of the type:

$$H_2 \mid HCl\ (m),\ S\ (x),\ H_2O\ (y) \mid AgCl\text{-}Ag$$

in which S is an added organic substance and x and y are the weight percents of the added substance and water respectively. The purposes of these studies are to determine the thermodynamic properties of the hydrochloric acid, to determine the value of the ion-size parameter of the acid in the various solutions, and to correlate the values of the standard cell potentials, E^0_m, with the dielectric constants of the solutions and the chemical natures of the added organic substances. The data obtained also serve to check the validity of the Debye-Huckel equations.

A list of the references to work on this type of cell was given by one of us in a paper presented before the National Bureau of Standards Symposium on Electrochemical Constants in 1951 [1]. Since 1951 additional data for the mixed solvent, water-methanol, have been published by Oiwa [2] and for D-fructose-water by Crockford, Little, and Wood [3]. In the work discussed in the present paper we are especially interested in the data for the following added organic substances: glycerol [4], D-fructose [3, 5], and D-glucose [6]. Other added substances that have been studied by various investigators are: dioxane, ethanol, propanol-2, and ethylene glycol [1].

In this paper the added substance was D-sorbitol and the concentrations used were 5 per cent and 20 per cent by weight. Acid concentrations ranged from approximately 0.01 m. to 0.3 m. All measurements were made at 25°C.

EXPERIMENTAL.—The procedures for purifying the chemicals, preparing the cells, measuring the densities and cell potentials, and calculating the vapor pressures were the same as those used by Crockford and Sakhnovsky [5]. All measuring equipment was properly calibrated. In the absence of dielectric constant data the corresponding D-glucose values as given by Williams, *et al.* [6] were used. These were 77.3 and 73.4 respectively for the 5 per cent and 20 per cent solutions. All electromotive force data were corrected to 1 atm. of hydrogen. The type of cell used was the same as that employed in previous work of this type in this laboratory [3, 4, 5, 6].

Values for the electromotive force are the averages of at least three cells. These usually agreed to ±0.05 mv. The times necessary for equilibrium were comparable to those found by Williams, *et al.* [6]. The results are given in international volts.

CALCULATIONS AND RESULTS.—The function, E', defined by Equation (1), was used in determining the standard cell potentials.

$$E' = E + 0.1183 \log m - \frac{0.1183A\sqrt{c}}{1 + aB\sqrt{c}} - 0.1183 \log (1 + 0.002mM_{xy}) \quad (1)$$

in which E' is the apparent molal potential, E is the observed electromotive force of the cell corrected to 1 atm. of hydrogen, m is the molality of the acid, A and B are the Debye-Huckel constants, c is the concentration of the acid in moles per liter, M_{xy} is the mean molecular weight of the solvent, and a is the ion-size parameter.

Table I gives the observed electromotive force values, the acid molalities, and the E' values calculated for an ion-size parameter of 6.6 A for the various solutions. Table II gives the values for the constants of Equation (1).

TABLE I

5% D-sorbitol solutions			20% D-sorbitol solutions		
m	E	E′	m	E	E′
0.008114	0.47222	0.22020	0.008197	0.41679	0.20980
.009961	.46206	.22017	.020128	.41815	.21008
.016059	.43832	.22000	.030061	.39871	.21006
.025309	.41630	.22005	.040131	.38486	.21012
.042780	.39088	.22006	.051388	.37293	.21006
.050798	.38261	.22006	.061576	.36425	.21004
.062296	.37285	.22012	.072460	.35850	.21006
.070069	.36708	.22000	.080328	.35145	.20993
.079785	.36080	.21995	.089225	.34623	.20970
.089862	.35526	.22010	.10198	.33961	.20944
.10080	.34956	.21988	.20051	.30658	.20843
.19996	.31602	.21896	.29854	.28663	.20725
.30194	.29551	.21799	—	—	—

TABLE II

Constants of equation (1)

	A	B	M_{xy}	a, A	D
5% D-sorbitol	0.52044	0.33105	18.87	6.6	77.3
20% D-sorbitol	0.56356	0.33995	21.98	6.6	73.4

The usual procedure for obtaining the best value for the ion-size parameter by plotting E' versus the molality for various values of this parameter was followed. Three such plots for the 20 per cent solution are shown in Figure 1. The value of 6.6 A gives a line of zero slope in the lower concentration range thus establishing it as the best value for the ion-size parameter. This same value was found for the carbohydrates, D-glucose [6] and D-fructose [3, 5]. It is higher than that found for other organic substances. This will be referred to later in the paper.

The values of the standard cell potentials are also established by the same plot. For the 5 per cent and 20 per cent solutions the values of E^0_m so obtained are 0.22006 and 0.21007 volt respectively. These are considered to be precise to ± 0.05 mv although the data used in the extrapolation tend to be erratic at 0.01 m of acid and in lower concentrations.

The density data fitted the following empirical equations:

$$d\ (5\%\ \text{solution}) = 0.0208\ m + 1.0147$$

$$d\ (20\%\ \text{solution}) = 0.0261\ m + 1.0715$$

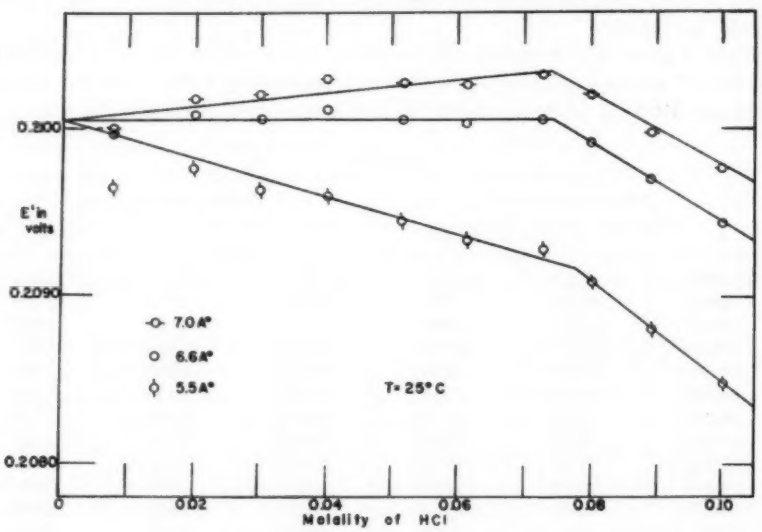

FIG. 1

TABLE III

Mean activity coefficients of hydrochloric acid in D-sorbitol-water solutions

Molality	5% D-sorbitol		20% D-sorbitol	
	Eq (2)	Eq (3)	Eq (2)	Eq (3)
0.01	0.905	0.905	0.898	0.895
.02	.879	.877	.866	.866
.03	.860	.859	.846	.846
.04	.845	.844	.830	.831
.05	.833	.833	.818	.819
.06	.823	.823	.808	.809
.07	.815	.815	.798	.800
.08	.808	.808	.795	.792
.09	.802	.801	.792	.785
.10	.797	.795	.788	.779
.20	.773	.756	.762	.738
.30	.763	.732	.755	.714

The mean activity coefficients, $\gamma_{\pm}$, of hydrochloric acid in the two solutions were calculated from the electromotive force data by the equation

$$\log \gamma_{\pm} = (E^0_m - E)/0.1183 - \log m \qquad (2)$$

The calculated values were plotted on a large scale and the values given in Table III were obtained at rounded molalities. The experimental values could be reproduced in all but the higher concentrations by the theoretical equation

$$\log \gamma_{\pm} = -\frac{A\sqrt{c}}{1 + aB\sqrt{c}} - \log (1 + 0.002\, mM_{sy}) \qquad (3)$$

The first systematic study of the standard cell potential as a function of the dielectric constant of the solution was made by Harned and Owen [7]. Their procedure consisted in plotting E^0_m versus $1/D$. Their considerations were extended by Crockford [1] to all new data available up to 1951. All considerations were limited to 25° data because of the lack of experimental work at other temperatures. Figure 2 includes all data considered by Harned and Owen, and Crockford, in addition to all data that have become available since 1951. This figure brings out a number of features. The points for the monohydric alcohols: methanol, ethanol, and propanol-2, as well as the points for dioxane fit roughly on a single curve. The glycerol data fit on another curve below that of the monohydric alcohols. The ethylene glycol data follow fairly closely the glycerol data at the lower concentrations but depart from the glycerol data at the higher concentrations.

In the summary by Crockford [1] in 1951 the data for the two carbohydrates, D-glucose and D-fructose, appeared to fall on the same curves located well below the glycerol curve. At that time data were available for only the 5 per cent and 10 per cent D-fructose solutions. Thus three more or less distinct sets of curves were indicated: one consisting of the carbohydrates, the second con-

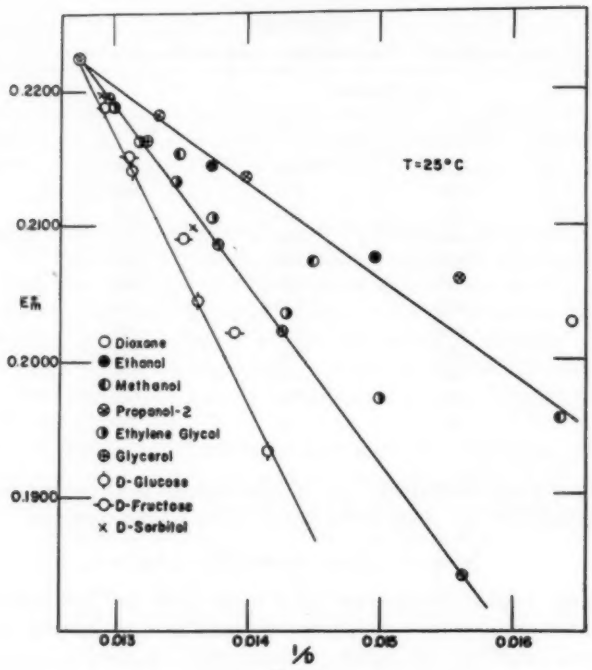

FIG. 2

sisting of glycerol and ethylene glycol, and the third consisting of the monohydric alcohols and dioxane. Thus considerable correlation seemed to exist between the positions of the three sets of curves and the chemical nature of the added substances. Additional data are now available for two higher concentrations of D-fructose [3] as well as the D-sorbitol data presented in this paper. In the case of methanol the data of Oiwa [2] made no appreciable change in the position of the methanol curve in the region studied.

When the new data are plotted, as seen in Figure 2, it is found that while the curve for D-fructose does not coincide with that of D-glucose, as originally indicated, it does lie close to it and the two can still be considered as making up a distinct set of curves. The data for D-sorbitol are seen to fall much closer to the glycerol curve than to the D-glucose and D-fructose curves. This is not surprising since D-sorbitol is an alcohol with a structure more complex than that of glycerol and at the same time it is a carbohydrate.

From the standpoint of the ion-size parameter only one clear-cut conclusion can be drawn. For the D-glucose, D-fructose, and D-sorbitol solutions the ion-size parameter of the acid with a value of 6.6 A is much larger than in the case of any other solvent pair and these three sets of data make up the three lower curves in Figure 2. In the case of the remaining solvent pairs the ion-size pa-

rameter varies from a value of 6.3 A in glycerol to 4.3 A in methanol, ethanol, and ethylene glycol solutions.

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HYDROGEN ION ACTIVITY EXPONENTS IN CITRATE AND PHOSPHATE BUFFER SOLUTIONS IN AQUEOUS METHANOL

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The purpose of the work presented in this paper was to determine the paH (negative logarithm of the hydrogen ion activity) in potassium dihydrogen phosphate-sodium hydroxide and potassium dihydrogen citrate-hydrochloric acid buffer systems using pure water and methanol-water mixtures as the solvent. The concentrations of methanol used were 10 per cent and 20 per cent by weight and all measurements were made at 25°C. The data obtained in such a study, besides being of interest in themselves, can be used for the determination of indicator constants in mixed solvents [1].

In this research electromotive force measurements were made with these two buffer systems in pure water and in the two methanol-water solutions using cells of the type:

$$\text{Pt, } H_2 \mid \text{Solution} \mid \text{AgCl-Ag}$$

From the data obtained the paH for each solution was calculated by means of the following equation:

$$\text{paH} = \frac{E - E_c^0}{0.05915} + \log C_{Cl^-} - \frac{A\sqrt{\mu}}{1 + aB\sqrt{\mu}} + C'\mu + \log \frac{d_0}{d + 0.001(M_1 - M_2)C_{Cl^-}} \quad (1)$$

in which C_{Cl^-} is the molarity of the chloride ion, A and B are the Debye-Hückel constants, a is the ion-size parameter, μ is the ionic strength, C' is the "salting-

out" constant, d_0 and d are the densities of the solvent and solution respectively, M_2 is the ionic weight of the chloride ion, and M_1 is the mean molecular weight of the solvent. E_c^0 is the standard cell potential expressed on the molarity scale. For a more detailed discussion of this equation the reader is referred to Masi and Knight [1].

EXPERIMENTAL.—The procedures for the purification of the various chemicals, the preparation of the solutions, and the preparation and use of the cells were the same as given by Masi and Knight [1]. The methanol was purified in the same manner as the ethanol in the reference. All measuring apparatus and equipment were properly calibrated and all electromotive force measurements were corrected to 1 atm. of hydrogen. The values of the electromotive force are the average of at least three cells agreeing to 0.04 mv. From three to five hours were required for the cells to come to equilibrium.

The concentration of monopotassium citrate was 0.0233 molal in all solutions and that of monopotassium phosphate was 0.0250 molal in all cases. All the phosphate solutions were made 0.00498 molal in potassium chloride to insure the proper functioning of the Ag-AgCl electrode. The molarities corresponding to these molalities are given in Table I.

CALCULATIONS.—The values for the constants of Eq (1) are summarized in Table II. The values of E_c^0, d_0, and M_1 are the same as those used by Harned and Thomas [2] in their studies with hydrochloric acid in water-methanol mixtures. The values of the Debye-Hückel constants, A and B, were calculated using the values for the dielectric constant given by these investigators. The values of the ion-size parameter are those calculated by Crockford [3]. The values for the "salting-out" constant were assumed to be the same as those used by Masi and Knight for their studies with ethanol. It should be noted that there can be a rather wide variation in this term without affecting the value of paH. The value for this "salting-out" constant in all the citrate buffer solutions is 0.13. For the phosphate solutions the value varies with the concentration

TABLE I

Molarity of added salts

	Monopotassium Citrate	Monopotassium Phosphate	Potassium Chloride
Pure water	0.0231	0.0249	0.00496
10% Methanol	0.0227	0.0245	0.00487
20% Methanol	0.0224	0.0241	0.00484

TABLE II

	E_c^0	A	B	a, B	M_1	D
Pure water	0.22154	0.5092	0.3286	5.6	18.02	78.9
10% methanol	0.21431	0.5556	0.3383	5.0	18.82	74.0
20% methanol	0.20692	0.6156	0.3501	5.0	19.86	69.2

TABLE III

Experimental and calculated data citrate buffers

Molarity (HCl)	Density	E	μ	paH
		Pure water		
0.00296	0.9989	0.58817	0.02322	3.611
.00593	.9989	.55877	.02338	3.416
.00889	.9989	.53722	.02361	3.228
.01185	.9990	.51962	.02395	3.055
.01481	.9990	.50334	.02450	2.893
.01776	.9990	.48856	.02560	2.703
.02073	.9991	.47435	.02874	2.529
.02369	.9991	.46139	.03250	2.364
.02666	.9991	.45485	.03500	2.305
		20% methanol		
0.00286	0.9665	0.59465	0.02251	4.086
.00574	.9665	.56559	.02267	3.898
.00860	.9666	.54385	.02290	3.706
.01147	.9666	.52575	.02324	3.526
.01434	.9666	.50845	.02379	3.331
.01720	.9666	.49088	.02486	3.114
.02007	.9667	.47392	.02786	2.898
.02293	.9667	.45717	.02840	2.674
.02580	.9667	.44275	.03000	2.483
		10% methanol		
0.00291	0.9819	0.59123	0.02289	3.771
.00583	.9819	.56187	.02304	3.577
.00874	.9819	.54040	.02326	3.389
.01166	.9819	.52205	.02360	3.204
.01456	.9819	.50552	.02414	3.020
.01747	.9820	.48960	.02522	2.829
.02039	.9820	.47356	.02825	2.623
.02330	.9820	.45890	.02928	2.432
.02621	.9820	.44608	.03050	2.265

of the buffer reagents. In only the two highest concentrations of sodium hydroxide is its value significant in the calculations. For the highest concentration of base the value used was 0.1 and for the next highest concentration the value was taken as 0.06. A discussion of this term is given by Masi and Knight.

The procedures for calculating the ionic strength in the various solutions were the same as those used by Masi and Knight. Since they are rather involved and lengthy they will not be reproduced here.

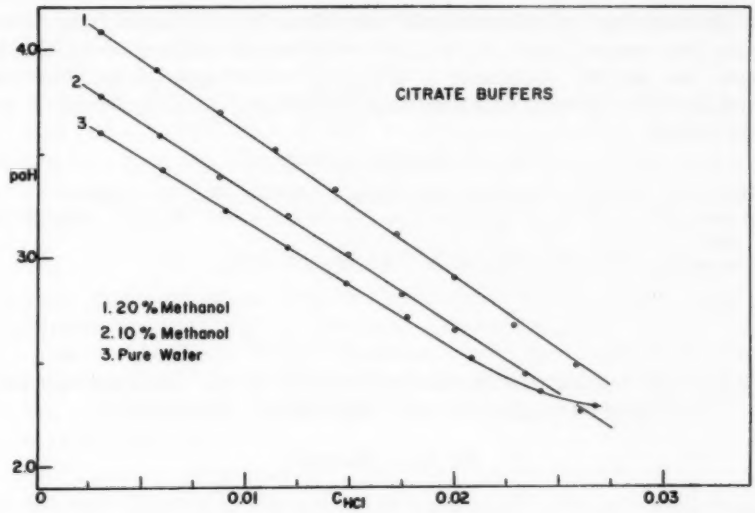
RESULTS.—As seen in Table III measurements were made with nine concentrations of hydrochloric acid ranging from approximately 0.003 M to 0.026 M. Table IV gives the results for phosphate buffer studies and it is noted that

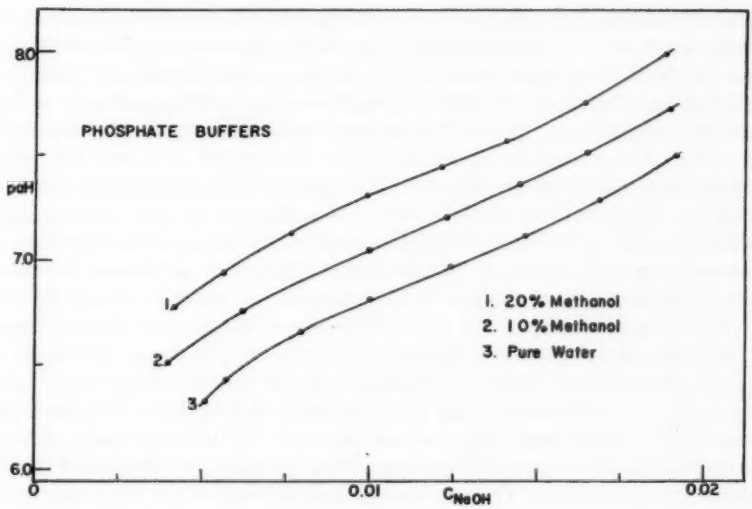
TABLE IV

Experimental and calculated data phosphate buffers

Molarity (NaOH)	Density	E	μ	paH
		Pure water		
0.004527	0.9994	0.73671	0.03892	6.332
.005660	.9996	.76361	.04119	6.447
.007925	.9997	.75547	.04572	6.645
.010190	.9999	.76548	.05025	6.811
.012457	1.0000	.77487	.05487	6.968
.014721	1.0001	.78429	.05932	7.124
.016998	1.0003	.79466	.06387	7.303
.019257	1.0004	.80723	.06838	7.515
		10% methanol		
0.004062	0.9827	0.74098	0.03749	6.510
.006088	.9827	.75390	.04155	6.725
.008123	.9827	.76427	.04562	6.898
.010148	.9827	.77285	.04967	7.040
.012232	.9827	.78189	.05383	7.190
.014461	.9827	.79144	.05829	7.349
.016636	.9827	.80186	.06264	7.527
.018900	.9827	.81458	.06717	7.742
		20% methanol		
0.004383	0.9670	0.75084	0.03771	6.792
.005473	.9670	.75776	.03989	6.906
.007663	.9674	.76963	.04427	7.102
.009850	.9675	.77953	.04864	7.267
.01204	.9676	.78885	.05302	7.421
.01423	.9677	.79825	.05740	7.578
.01642	.9677	.80847	.06178	7.752
.01861	.9678	.82101	.06615	7.972

eight concentrations of sodium hydroxide were used ranging from approximately 0.004 M to 0.02 M. Tables III and IV besides giving the molarity of the hydrochloric acid and sodium hydroxide, respectively, also give the densities of the various solutions, the electromotive force values for the cell, the ionic strength of the various solutions, and the calculated values of the paH. The figures show the curves obtained when the various values of the paH are plotted versus those of the concentration of the hydrochloric acid or the sodium hydroxide for the two buffer systems. It is noted that the paH values are in the acid range for the citrate buffers and fall both below and above a paH of 7.0 with the phosphate buffer system. In both cases the paH increases in value with an increase in the concentration of the methanol. This increase in concentration is of course accompanied by a decrease in the dielectric constant of the solution.





SUMMARY

1. Electromotive force measurements have been made at 25°C. on the cell,

$$Pt, H_2 \mid \text{solution} \mid AgCl\text{-}Ag,$$

in solutions in which the solvent was pure water, or water containing 10 per cent and 20 per cent methanol by weight.

2. Hydrogen ion activities and paH values have been calculated from electromotive force measurements in the above solvents on solutions of varying hydrogen ion activity containing a potassium dihydrogen phosphate-sodium hydroxide buffer system, or a potassium dihydrogen citrate-hydrochloric acid buffer system.

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ANALYTIC THOMAS-FERMI CALCULATION OF THE MAGNETIC SUSCEPTIBILITY OF DIATOMIC NITROGEN

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1. INTRODUCTION.—The Thomas-Fermi model [7, 4], so frequently used for atoms, can be set up for molecules as well, but the labor involved in extracting information from the molecular model has frequently been considered inappropriate in view of the approximate nature of the system description. Hund's [6] statistical calculation of the electron distribution in a Thomas-Fermi nitrogen molecule is well known, but the complications encountered in this situation do not constitute a strong recommendation for the method as a tool for routine investigation of molecular structure. The approximate analytic solutions obtained by Brinkman [2] in his recent reconsideration of the Thomas-Fermi problem provide a remarkable simplification of the computation involved. In demonstrating the success of this treatment, Brinkman indicated the electric potential of nitrogen on the axis of the molecule as found by Hund [6] to be "... indistinguishable from our curve, if drawn on the scale of Hund's figure." [2] Brinkman and Peperzak [3] have noted, however, that for any molecule this approximation always leads to a value of zero for the dipole moment. Actual computation of a measurable bulk property seems desirable for full appreciation of the utility of Brinkman's approximate solution. The obvious choice of molecular system for such a computation is diatomic nitrogen, for there exist the Ritz variation solutions of the Thomas-Fermi equation worked out by Hund for that system and the calculation of magnetic susceptibility from the Hund electron density as reported by Bonet and Bushkovitch [1]. The purpose of this paper is to calculate the magnetic susceptibility of molecular nitrogen from Brinkman's analytic distribution functions in order to effect a comparison with the Hund result.

2. ANALYTIC DISTRIBUTION FUNCTIONS.—Electron distribution functions for molecular nitrogen are obtained by solving the Thomas-Fermi equation for the potential V. The mathematical condition is $\nabla^2 V = (8/3\pi a_0)(2/ea_0)^{1/2}V^{3/2}$,

where a_0 is the first Bohr radius and e is the protonic charge. For molecular nitrogen, the equation is rewritten:

$$x_1^{-1}\frac{d^2F(x_1)}{dx_1^2} + x_2^{-1}\frac{d^2F(x_2)}{dx_2^2} = [x_1^{-1}F(x_1) + x_2^{-1}F(x_2)]^{3/2}$$

where $x_i = r_i[(2/ea_0)(4/3\pi)^{2/3}Z^{1/3}]^{-1}$, r_i is the distance from a point in space to the ith nucleus, Z is the charge of a nitrogen nucleus, and $F(x)$ is a function such that

$$x_1^{-1}F(x_1) + x_2^{-1}F(x_2) = (V/Ze)(2/ea_0)(4/3\pi)^{2/3}Z^{1/3}$$

Brinkman [2] has shown that $F(x) \backsim C_1x^{1/2}K_{2/3}(C_2x^{3/4})$ where $K_{2/3}$ is a modified Bessel function and that C_2 has the value 1.09 for nitrogen.

In order to obtain the electron distribution from the results of Brinkman's work, one need only employ Poisson's equation to identify $\nabla^2V/4\pi e$ or $(3\pi^2)^{-1}(2V/ea_0)^{3/2}$ as the density of electrons. In terms of the reduced distances x_i, the electron density is

$$\rho = (C_1Ze^{3/2}/4\pi)\{x_1^{-1/2}K_{2/3}(1.09x_1^{3/4}) + x_2^{-1/2}K_{2/3}(1.09x_2^{3/4})\}^{3/2} \tag{1}$$

The behavior of the density function for large values of x_i is quite proper. Noting that $K_p(u) = \pi(2 \sin \pi p)^{-1}[I_{-p}(u) - I_p(u)]$ for non-integral values of p and that $I_p(u) = i^{-p}J_p(iu)$, one can see that $K_p(u)$ is a monotonically decreasing function which approaches the u axis asymptotically. All that really must be required of $K_{2/3}(u)$ is that it not become infinite for large u, since the factor $u^{-1/2}$ in the density would then make sure that the density vanishes when u becomes large without limit. The function $u^{-1/2}K_{2/3}(u^{3/4})$ has a singularity at the origin.

The fact that the density goes as the three halves power of the sum of the $F(x_i)/x_i$ introduces a complication in the evaluation of average values with density used as a distribution function. The problem is easier to see when transformation is made to prolate spheroidal coordinates with the foci taken as nuclear locations. Then $r_1 = a(\xi + \eta)$ and $r_2 = a(\xi - \eta)$ with $2a$ taken as the nuclear separation. This gives $x_1^{-1}F(x_1) \sim (\xi + \eta)^{-1/2}K_{2/3}[k(\xi + \eta)^{3/4}]$ and $x_2^{-1}F(x_2) \sim (\xi - \eta)^{-1/2}K_{2/3}[k(\xi - \eta)^{3/4}]$. The normalization of the electron distribution function corresponds to calculation of the total charge as $14e$. Since the element of volume is $a^3(\xi + \eta)(\xi - \eta)\,d\xi\,d\eta\,d\phi$ and since $-1 \leq \eta \leq 1$ and $1 \leq \xi$,

$$\text{total charge} = ea^3\int_1^\infty\int_{-1}^{1}\rho(\xi,\eta)(\xi - \eta)(\xi + \eta)\,d\eta\,d\xi \tag{2}$$

The integrand has a singularity at nucleus 1, where $\xi = -\eta = 1$, and at nucleus 2, where $\xi = \eta = 1$. Glazer and Reiss [5] have noted essentially the same problem in their relaxation calculations. To avoid the difficulty, one creates a boundary around these points, which means that a small volume about each nucleus is excluded from consideration in the integration over space. (2) can be rewritten

$$\text{total charge} = 2ea^3\int_1^\infty\int_0^{\eta\max}\rho(\xi,\eta)(\xi - \eta)(\xi + \eta)\,d\eta\,d\xi \tag{3}$$

with $\eta_{max} = \xi - 0.1$ for $1 \leq \xi \leq 1.1$ and $\eta_{max} = 1$ for $\xi > 1.1$. The artificial nature of this boundary condition must be considered in the evaluation of the constant C_1 appearing in (1). C_1 is determined so that the potential behaves like r_2^{-1} near nucleus 2 or such that $\lim r_2 V = Ze$ as r_2 approaches zero. This limit can also be written $\lim [F(x_2) + x_2 x_1^{-1} F(x_1)] = 1$ as x_2 approaches zero. Setting $F(x_2) + (x_2/x_1)F(x_1) = 1$ at the boundary ($\eta = 0.9$ and $\xi = 1$), one immediately finds the value 2.244 for C_1.

Bonet and Bushkovitch [1] have pointed out the necessity for using a model with an outer bounding surface beyond which the charge distribution vanishes. They determined this surface for homonuclear diatomic molecules as that on which the electron density is $0.003074/a_0^3$. Since the coordinate surfaces are those of prolate spheroids, η varies with ξ over the surface. Thus the η integration range for $\xi = 3.5$ must be 0.6 to 1 in order to apply this outer boundary condition. For $\xi = 4$, one finds that the density vanishes for all values of η between 0 and 1. The boundary conditions are very easy to apply in actual numerical integration of expressions such as (3).

Evaluation of the total charge by integration from the inner to the outer boundary as described above showed that very little charge was excluded in the process of setting up the inner boundary. The calculated charge is 99.99 per cent of $14e$.

3. SUSCEPTIBILITY CALCULATIONS.—The diamagnetic part of the susceptibility of molecular nitrogen is given by the well-known formula $-N_0(e^2/6mc^2)\overline{r^2}$ with $\overline{r^2}$ given as $\int \rho r^2 \, d\tau$. In the elliptic coordinates previously employed, $r^2 = a^2(\xi^2 + \eta^2 - 1)$ and

$$\overline{r^2} = a^5 \int\int \rho(\xi, \eta)(\xi^2 + \eta^2 - 1)(\xi^2 - \eta^2) \, d\eta \, d\xi$$

With the previously discussed boundary values for ξ and η, the straightforward but tedious numerical integration yields as the value of the diamagnetic contribution to the susceptibility -22.0×10^{-6}. The paramagnetic contribution to the susceptibility is given by $(N_0 e^2/6m^2c^2h)\overline{P^2}/\nu'$[1] in terms of $\nu' = 2.74 \times 10^{15}$ sec^{-1} and $\overline{P^2}$, the mean square of the total angular momentum. The expression for $\overline{P^2}$, $\int P^2 R(P^2) \, dP^2 \Big/ \int R(P^2) \, dP^2$ with

$$R(P^2) = \int_1^{\xi_0} \int_0^1 \left[\left(\frac{3h^3 \rho}{8\pi} \right)^{2/3} - \frac{P^2}{4a^2(\xi^2 + \eta^2 - 1)} \right]^{\frac{1}{2}} \frac{(\xi^2 - \eta^2)}{(\xi^2 + \eta^2 - 1)} \, d\eta \, d\xi$$

can be evaluated by triple numerical integration in the standard fashion. Using the density as given in (1), with x_i expressed in elliptic coordinates, one obtains for the paramagnetic contribution to the susceptibility 1.9×10^{-6}. Combination with the diamagnetic term gives for the total susceptibility of nitrogen a value -20.1×10^{-6}.

Comparison of the above values with those obtained by Bonet and Bushkovitch from Hund's electron distribution is quite interesting for two reasons.

First, the individual contributions to the total susceptibility are considerably smaller than for the Hund values (-33.7×10^{-6} and 9.1×10^{-6}), and the total susceptibility obtained from the distribution in (1) is lower than the Hund value (-24.6×10^{-6}). Second, the total susceptibility lies between the Hund value and the measured value. The fact that the result of the analytic distribution calculation is closer to experiment (-13.3×10^{-6}) than is the Hund value should not by itself prompt any suggestion of fundamental superiority of the analytic distribution; error cancellation has helped give a slightly lower result. The important conclusion to be drawn from the above work is that the Brinkman solution provides for the Thomas-Fermi electron distribution function an approximation which appears to be useful in the calculation of certain molecular properties and which may effect a very great saving of labor in such calculations.

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LACK OF SENSITIVITY TO BASE-CATALYSIS OF THE REACTIONS OF AMINES WITH 1-SUBSTITUTED-2,4-DINITROBENZENES

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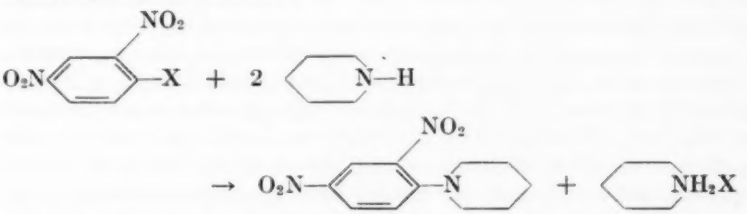
Primary and secondary amines are effective nucleophilic reagents and as such can effect the displacement of halogen atoms and other substituents of potential anionic stability from suitable aromatic systems. For example, piperidine reacts with many 1-substituted-2,4-dinitrobenzenes to form 2,4-dinitrophenylpiperidine (equation 1).



(1)

Such a reaction comprises the following specific processes: formation of a

[1] At present with the United States Air Force.

C—N bond, breaking of a C—X bond, and removal of a proton from the nitrogen atom. Much interest attaches to the question of the order in which these processes occur. Bunnett, Garbisch and Pruitt [1] found that several 1-substituents (X groups), representing different elements, were displaced according to equation 1 at nearly the same rate; this showed that the C—X bond is essentially intact in the transition state of the rate-determining step and strongly suggested that the C—N bond is formed before the C—X bond is broken.

Two items of evidence suggested that proton removal from nitrogen does not precede the rate-determining step: the fact that piperidine and N-deuteropiperidine react at equal rates with o-chloronitrobenzene, or with its para-isomer, in xylene solution [2], and the fact that the reaction of piperidine with 2,4-dinitrochlorobenzene is first-order and not a higher order in piperidine [3]. Did proton-removal from nitrogen precede the rate-determining transition state, piperidine would act as an effective proton-acceptor and therefore would be involved in the kinetic expression for the reaction to a power greater than unity. Nevertheless, it seemed desirable to test further the proposition that proton-removal follows the rate-determining step by determining the kinetic effect of an added strong base. If the proposition were correct, the reaction would not be catalyzed by a strong base.

The effect of added sodium hydroxide on the rates of reactions represented by equation 1 was studied. Sodium hydroxide was chosen because, though a strong base, it is weakly nucleophilic compared with piperidine [4], and thus the side reaction of displacement of the 1-substituent by the base could be minimized. Fifty per cent dioxane was chosen as solvent in large part because it cannot undergo chemical change by reacting with hydroxide ion; alcoholic solvents interact with hydroxide ion to form alkoxide ions which are relatively strong nucleophilic reagents. In the kinetics runs, both the amine and hydroxide ion were present in large excess with respect to the aromatic substrate. For example, in many runs the substrate was 4×10^{-4} M, piperidine was 1.6×10^{-2} M (40-fold excess) and sodium hydroxide (if used) was 1.6×10^{-2} M. Under these conditions, pseudo-first order kinetics were achieved, with resultant simplification of the mathematical treatment of the data.

The main reaction product, 2,4-dinitrophenylpiperidine, is orange whereas the by-products (including 2,4-dinitrophenol, the product of displacement of a 1-substituent by hydroxide ion) are colorless in solutions of moderate acidity. It is therefore easy to follow the progress of the reaction by quenching aliquots of the reaction mixture in a standard volume of dilute acid and measuring the optical density of the quenched solutions at a wave length such as 380 mμ. The optical density of an "infinity" sample, similarly quenched, is also measured, and the slope of a plot of $\log_e$ (O.D.$_\infty$ − O.D.) vs. time is the total first order rate coefficient for all reactions consuming the substrate. The yield of 2,4-dinitrophenylpiperidine is easily determined by comparing the infinity optical density against the optical density of a quenched aliquot of a mock infinity solution (a solution containing 2,4-dinitrophenylpiperidine in the concentration expected on the assumption that this product was formed quantitatively). The total first order coefficient is multiplied by the fractional yield of 2,4-dinitro-

phenylpiperidine to get the first order rate coefficient for the reaction forming this product; the difference between this coefficient and the total first order coefficient is the first order coefficient for the reaction with hydroxide ion. Each first order coefficient is then divided by the concentration of the relevant nucleophilic reagent to obtain the second order rate coefficient. This calculation of rate coefficients is expressed concisely in the following equations:

$$-\log_e (\text{O.D.}_{\infty,\text{exp}} - \text{O.D.}) = k_{\text{tot}}t + \text{constant}$$

$$k_{\text{pip}} = (\text{O.D.}_{\infty,\text{exp}}/\text{O.D.}_{\infty,\text{theo}})k_{\text{tot}}$$

$$k_{\text{OH}^-} = k_{\text{tot}} - k_{\text{pip}}$$

$$k'_{\text{pip}} = k_{\text{pip}}/[\text{piperidine}]$$

$$k'_{\text{OH}^-} = k_{\text{OH}^-}/[\text{OH}^-]$$

In these equations, k_{pip}, k_{OH^-}, and k_{tot} are, respectively, first order rate coefficients for reactions of the substrate with piperidine and with hydroxide ion, and for the total of all reactions consuming the substrate. k'_{pip} and k'_{OH^-} are second order rate coefficients. $\text{O.D.}_{\infty,\text{theo}}$ is the infinity optical density expected on the assumption of a quantitative yield of 2,4-dinitrophenylpiperidine, $\text{O.D.}_{\infty,\text{exp}}$ is the actual (experimental) infinity optical density, and O.D. is the optical density of a quenched sample removed from the reaction at time t.

RESULTS AND DISCUSSION.—Reactions of piperidine with four substrates were studied: 2,4-dinitroiodobenzene, 2,4,4′-trinitrodiphenyl ether, 2,4-dinitrodiphenyl sulfone and 2,4-dinitrochlorobenzene. In addition, the reaction of aniline with 2,4-dinitrochlorobenzene was investigated. All our rate measurements are presented in Table I.

It will be noted that there is some variation in rate coefficients for nearly identical runs, owing to normal experimental error. Added lithium chloride and piperidine hydrochloride have no significant effect on the reaction rate with any substrate; this shows the absence of kinetic salt effects and that the reactions are not acid-catalyzed, since the piperidinium ion is an acid.

With all substrates but one, the rate of formation of 2,4-dinitrophenylpiperidine is not increased by added sodium hydroxide. (The apparent slight increase in some cases is within experimental error.) This, which is the most significant result from this work, shows that *proton removal from nitrogen does not occur before or during the rate-determining step* in any of these reactions. Hydroxide ion is a much stronger base than any other component of the reaction mixture and would cause a large increase in rate did the reaction depend on the removal of a proton from any atom prior to the rate-determining transition state. Specifically, this observation excludes the following conceivable mechanisms: (a) removal of a proton from piperidine to form piperidide ($C_5H_{10}N^-$) ion, followed by attack of piperidide ion on the aromatic substrate; (b) concerted termolecular displacement in which formation of the C—N bond, rupture of the C—X bond and removal of the proton from nitrogen all occur in one process; and (c) a two-step, intermediate complex mechanism in which the C—N bond forms initially but reversibly and removal of the proton from nitrogen is necessary

TABLE I

Reactions of piperidine with 1-substituted-2,4-dinitrobenzenes in 50% aqueous dioxane at 0°C.

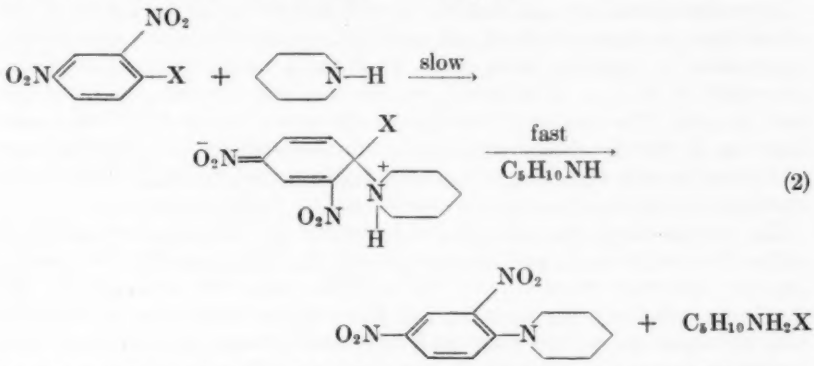
1-Substituent	Initial Concentrations, moles/l.			Second order rate coefficients, l. mole^{-1} min.$^{-1}$, for reaction with	
	Substrate	Piperidine	Other substance	$C_5H_{11}N$	OH^-
I	0.00100	0.0402		0.216	
	.00100	.0402		.214	
	.00100	.0402	LiCl, 0.0392	.214	
	.00100	.0402	LiCl, .0392	.213	
	.00050	.0200		.228	
	.00050	.0200	$C_5H_{10}NH_2Cl$, 0.020	.226	
	.00050	.0200	NaOH, 0.0200	.234	0.0025
$-OC_6H_4NO_2-p$	.00040	.01607		1.35	
	.00040	.01607		1.39	
	.00040	.01607	LiCl, 0.0157	1.40	
	.00040	.01600		1.26	
	.00040	.01600		1.22	
	.00040	.01600	$C_5H_{10}NH_2Cl$, 0.016	1.28	
	.00040	.01600	NaOH, 0.0160	1.60	.0175
$-SO_2C_6H_5$	.000414	.01663		.936	
	.000414	.01663		.985	
	.000414	.01663	LiCl, 0.0157	1.000	
	.000501	.02006		.891	
	.000501	.02006	$C_5H_{10}NH_2Cl$, 0.020	.876	
	.000501	.02006	NaOH, 0.0200	.886	.15
Cl	.000401	.01608		.829[a]	
	.000500	.0200		.828[a]	
	.000500	.0200	$C_5H_{10}NH_2Cl$, 0.020	.835	
	.000500	.0200	$C_5H_{10}NH_2Cl$, .040	.853	
	.000500	.0200	NaOH, 0.0200	.840	.043
	.000500	.0200	NaOH, .0400	.860	.020
For reaction with aniline instead of piperidine, at 45.95°					
Cl	0.000451	0.427		0.0244	
	.000451	.427	NaOH, 0.0168	.0240	0.371
	.000451	.427	NaOH, .0352	.0252	.383

[a] J. F. Bunnett and R. J. Morath, Jour. Amer. Chem. Soc., **77:** 5165 (1955), reported k = 0.821 l. mole^{-1} min.$^{-1}$; their value was determined by titration of chloride ion formed during the reaction.

before the C—X bond can be broken. Insofar as elimination of the second and third possibilities is concerned, the present work reenforces the conclusions of Bunnett, Garbisch and Pruitt to which reference was made in the introduction of this paper.

The present results, together with those of Bunnett, Garbisch and Pruitt [1], indicate that these reactions occur by the intermediate complex mechanism

with the first step rate-determining. In this mechanism, shown in equation 2, the C—N bond is formed in an initial slow step and



(2)

the intermediate complex so formed decomposes rapidly with loss of X from carbon and a proton from nitrogen. The chronological order of the latter two processes is not revealed by evidence now available.

The reaction of aniline with 2,4-dinitrochlorobenzene is also insensitive to catalysis by sodium hydroxide, and the same conclusions can be drawn with respect to this reaction. The reaction of piperidine with 2,4,4′-trinitrodiphenyl ether (in which the p-nitrophenoxy group is displaced), on the other hand, does appear to go 14–29 % faster in the presence of sodium hydroxide. This is a small effect and its interpretation is not obvious, although mechanism (c) above is suspected.

Mechanism (a) which is excluded, except perhaps for the reaction of piperidine with 2,4,4′-trinitrodiphenyl ether, by our results is similar to the mechanism proposed by Betts and Hammett [5] and adopted by Watanabe and DeFonso [6] to explain base-catalysis of the reactions of ammonia and n-butylamine with carboxylic esters. Our results show that if hydroxide ion is able to convert piperidine to piperidide ion, the concentration and/or nucleophilic reactivity of the latter are so low that no significant part of the production of 2,4-dinitrophenylpiperidine in the presence of hydroxide ion occurs via this mechanism. It seems unlikely, then, that in the alcoholic solvents used for the ester aminolysis studies the amount of amide ions formed could be so much larger, or that the amide ion: amine rate ratio for reaction with an ester could be so much greater, as to account for the production of a large fraction of the carboxylic amide product by mechanism (a). Doubts are therefore raised about the validity of the mechanism proposed by Betts and Hammett.

Brady and Cropper [7] found that second-order rate coefficients for the reaction of methylamine with 2,4-dinitrochlorobenzene in ethanol increased with increasing methylamine concentration or with increasing concentration of added triethylamine. They interpreted the effect as base-catalysis, but our present rigorous exclusion of base-catalysis for very similar cases renders their interpretation untenable. The amine concentrations in their reaction solutions were

quite high (0.3 to 1.2 molar) and variations in rate coefficients with varying amine concentration are reasonably ascribed to general medium effects.

Apart from their principal significance with respect to the mechanism of the substitution, as discussed above, our results allow some interesting quantitative comparisons of reactivity to be made. First, the sameness of the rates of displacement of the four substituents, representing four elements, by piperidine may be noted. The maximum variation in rate is less than six-fold. The similar sameness of rate for these very reactions in methanol solution was the basis of the mechanistic argument of Bunnett, Garbisch and Pruitt [1]; the present results show that this result is not coincidental for a single solvent.

The comparatively rapid reaction of hydroxide ion with 2,4-dinitrodiphenyl sulfone is perhaps due in part to displacement of a nitro group [8]. The remaining three substrates react at more or less similar rates with hydroxide ion, the maximum variation being about ten-fold. Since the rate coefficients for reactions with hydroxide are derived from small differences between large numbers, they are better regarded as estimates than as significant data.

Comparison of data in Table I for reactions in 50 per cent dioxane with data of Bunnett, Garbisch and Pruitt for the same reactions in methanol, all at 0°, shows that with all four substrates 50 per cent dioxane is the faster solvent. The 50 per cent dioxane: methanol rate ratios are, according to the substituent displaced, as follows: Cl, 7.1; I, 8.1; $SO_2C_6H_5$, 10.9; $OC_6H_4NO_2$-p, 16.0.

Under the conditions used for the reactions of 2,4-dinitrochlorobenzene with aniline *plus* sodium hydroxide, hydrolysis and aminolysis of the aryl chloride occurred at roughly equivalent rates; therefore, the rate coefficients in Table I for reactions both with aniline and hydroxide ion at 45.95° are quite precise. Comparison of the present hydroxide value, 0.377 l. mole^{-1} min.$^{-1}$ in 50 per cent dioxane, with a value extrapolated from data of Bunnett and Davis [4], 0.453 l. mole^{-1} min.$^{-1}$ in 60 per cent dioxane, shows that the rate increases with change to a *less* polar solvent. Similar effects have been noted by other investigators who found that the reaction of 2,4-dinitrochlorobenzene with sodium methoxide is faster in benzene-methanol [9] or in methyl acetate-methanol [10] than in pure methanol. The present value for reaction with aniline, 0.0244 l. mole^{-1} min.$^{-1}$ in 50 per cent dioxane, compares with Davis' [11] coefficient, 0.0182 l. mole^{-1} min.$^{-1}$ in 60 per cent dioxane. This reaction is faster in the *more* polar solvent.

EXPERIMENTAL. *Materials.*—The four aromatic substrates and piperidine hydrochloride were available from the earlier investigation [1]. Dioxane was purified by refluxing the commercial product several hours with lithium aluminum hydride and then four hours with sodium metal, with subsequent distillation through a good column; material boiling at 100.4–100.6° was taken for use in rate experiments. Fifty per cent dioxane was prepared by mixing equal volumes of purified dioxane and distilled water. Commercial piperidine was refluxed six hours over sodium metal and then distilled from sodium metal. Aniline was redistilled from zinc dust before use.

Kinetics measurements.—The general procedure of Bunnett, Garbisch and Pruitt [1] was used. Photometric measurements were made at 380 mμ for re-

actions with piperidine and at 365 mμ for reactions with aniline. In the latter a small correction was made for absorption due to 2,4-dinitrophenol in runs containing sodium hydroxide. In the former, such a correction would have been negligible. For runs containing sodium hydroxide, the treatment of the data was modified somewhat as discussed in the text of this article.

SUMMARY

Added sodium hydroxide does not accelerate reactions of piperidine with three 1-substituted-2,4-dinitrobenzenes or of aniline with 2,4-dinitrochlorobenzene. This result shows that removal of a proton from the nitrogen atom of the amine does not occur prior to the rate-determining transition states of these reactions. The intermediate complex mechanism for these reactions is thus further supported.

ACKNOWLEDGMENTS

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THE INFLUENCE OF DIET ON GLUCURONIDE EXCRETION[1]

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Urinary glucuronides have recently been implicated in the problem of the calcium type of urolithiasis. Prien and Walker (1955, 1956) have reported some

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degree of success in treating a limited number of patients who have a history of recurring urinary stones of the calcium type by the administration of salicylates in the form of acetylsalicylic acid or salicylamide. They attribute this success to a greater solubility of calcium in urine brought about by the increased excretion of glucuronides following salicylate administration. The increase in glucuronide excretion elicited by salicylate administration may also be dependent upon other factors in addition to the simple detoxication of salicylates. That this is the case has been demonstrated by Tseng, *et al.* (1950). These investigators found that the simultaneous administration of salicylates and sodium bicarbonate brought about a decrease in glucuronic acid excretion by comparison with data for patients receiving only salicylates. Other metabolic factors may also be involved in glucuronide formation since Saltzman, *et al.* (1954) have reported elevated serum glucuronic acid levels in diabetes and liver disease.

The decreased glucuronide excretion in the case of the simultaneous administration of salicylates and bicarbonate raises the question of whether glucuronide excretion may not be related to the acid or alkali content of the diet, as is the case with some of the other organic acids such as citrate (Schuck, 1934). With this question in mind, it was decided to investigate the effect of diet upon urinary glucuronide excretion.

MATERIALS AND METHODS.—The 24 hour urinary excretion of total glucuronic acid was followed on 55 medical students on their normal self-selected diets. These students were then divided into groups of varying size and their glucuronide excretion determined while they were on an assigned diet.

Composition of the diets. High protein diet: A diet made up primarily of meats, cheese, eggs, milk and other dairy products, plus a high protein snack before retiring at night.

Low protein diet: Essentially a high carbohydrate diet from which meat, eggs, cheese, milk and leguminous vegetables were excluded. Intake of bread was held to a minimum.

Fasting diet: First day—normal diet; second and third days—complete fast. Subject was allowed to have water *ad lib.*

Acid diets: a. Ammonium chloride: First day—normal diet; second day—normal diet plus 1 gm. of ammonium chloride at each meal; third day—normal diet plus 1 gm. of ammonium chloride at each meal, mid-morning, mid-afternoon, and at bedtime.

b. Prunes: First day—normal diet; second day—normal diet plus 2 dozen prunes; third day—normal diet plus at least 4 dozen prunes spread throughout the day. The acid quality of this diet depends upon the presence in prunes of organic acids not metabolized in the body.

Alkaline diets: a. Citrus fruits: First day—normal diet; second day—normal diet plus six oranges at night; third day—normal diet plus two to three dozen oranges distributed throughout the day.

b. Sodium citrate: First and second days—normal diet; third day—normal diet plus 10 gm. of sodium citrate before each meal.

TABLE I

Glucuronic acid excretion by humans on various diets

Subject	Normal Urine		Experimental Urine		Subject	Normal Urine		Experimental Urine	
	pH	Glucuronic Acid mg./24 hr.	pH	Glucuronic Acid mg./24 hr.		pH	Glucuronic Acid mg./24 hr.	pH	Glucuronic Acid mg./24 hr.
High Protein Diet					**Prunes Diet**				
CET	6.2	208	5.8	358	JSG	6.2	270	5.1	492
BSR	6.4	663	6.0	336	WDL	6.5	189	5.5	168
NAD	5.8	216	5.8	264	RMF	7.0	446	5.3	289
ASM	6.5	385	6.0	363	BCB	6.7	278	6.0	215
JE	6.0	216	5.7	272	ELM	6.3	234	5.4	460
TCR	6.4	360	5.3	383	ELS	6.3	286	5.6	434
JAK	6.3	523	5.7	683					
CM	6.3	422	6.1	461					
Low Protein Diet					**Sodium Bicarbonate Diet**				
BBW	5.8	425	6.5	259	RMS	5.8	351	7.3	195
TRW	7.1	400	6.1	623	DJH	6.9	346	7.7	319
FSJ	6.6	260	5.9	479	JBG	5.9	238	7.9	182
WMG	5.7	419	6.5	333	WRR	6.3	397	7.8	363
CFG	6.4	527	6.4	330	DSM	6.4	556	7.8	308
FMH	5.5	587	6.4	297	RCB	6.2	366	7.5	320
DP	6.5	272	5.7	372	GWG	5.9	263	8.1	280
MMW	5.6	275	6.2	495					
Fasting Diet[1]					**Sodium Citrate Diet**				
CRL (1)	5.7	276	5.6	189	BWB	5.4	333	7.7	219
(2)	—	—	6.2	251	JCT	6.1	113	7.6	529
ATJ (1)	6.8	259	5.6	175	DLK	6.2	120	7.3	238
(2)	—	—	5.8	148	RCW	6.4	543	6.7	425
JDB (1)	7.1	290	5.8	166	AED	5.8	205	8.0	385
(2)	—	—	5.6	171	HC	5.9	364	7.4	155
OJH (1)	5.7	356	5.2	255	ECL	5.7	110	7.9	204
(2)	—	—	5.3	181	MCG	6.2	285	8.1	419
MKK (1)	6.2	698	5.2	289					
(2)	—	—	5.5	133					
Ammonium Chloride Diet					**Citrus Fruit Diet**				
JK	7.2	319	5.5	293	RLG	5.7	677	6.7	698
KBL	6.4	586	5.9	354	WTH	6.2	382	6.2	523
RFS	6.5	201	5.8	369	DLH	5.6	231	6.9	566
BT	6.7	200	5.3	553	MAJ	5.9	199	6.4	480
CF	6.8	527	5.9	194	DRL	6.8	234	6.0	861
TS	7.5	357	6.3	278	CCW	6.0	518	6.9	1045
DHR	6.5	261	5.8	259					

[1] The numbers in parentheses indicate number of days on the fast.

TABLE II

Glucuronic acid excretion by humans on normal diet supplemented with vitamin C

Subject	Normal Urine		Experimental Urine	
	pH	Glucuronic Acid mg./24 hr.	pH	Glucuronic Acid mg./24 hr.
CFG	6.4	527	6.6	333
DJH	6.9	346	5.6	252
GWG	5.9	263	5.6	315
JE	6.0	216	5.5	384
ELS	6.3	286	6.3	531
ES	5.8	198	5.9	389
CRL	5.7	276	6.0	394
JDB	7.1	290	6.2	379

c. Sodium bicarbonate: First and second days—normal diet; third day—normal diet plus 10 gm. of sodium bicarbonate before each meal.

In each case where the normal diet was supplemented with the constituents listed above, the subjects were required to maintain a diet conforming as closely as possible to that consumed during the time of the collection of the normal urine sample. All diets covered a three day period unless otherwise noted. On the last day of the dietary period, 24 hr. urine collections were made and preserved over chloroform. No attempt was made to prevent loss of carbon dioxide, therefore the pH values for the urines in the pH 8 range may be somewhat in error. Subjects on the fasting diets were required to collect 24 hr. urine samples on each day of the fast.

Urinary pH determinations were made with the Beckman model G pH meter using the glass electrode. Total urinary glucuronic acid was determined by the method of Fishman and Green (1955). This determination depends upon the reaction of glucuronic acid with naphthoresorcinol to give a violet color.

RESULTS.—The data for the 24 hour excretion of glucuronides by humans on various dietary regimens are shown in Table I. Examination of the data in Table I reveals no apparent correlation between the acid or alkali content of the diet and the glucuronide excretion obtained. The only diets which produced definite changes in glucuronide excretion were the fasting diet and the citrus fruit diet. On the fasting diet the glucuronide excretion fell to the quite low levels of 133–255 mg./24 hrs. as compared with the normal range of 200–600 mg./24 hrs.

On the citrus fruit diet an elevation of glucuronide excretion was noted. Except in one case this increase was of the order of two to three times the output of these individuals on their normal diet. The question immediately arose concerning the possibility that this increase might be associated with the vitamin C content of the oranges consumed in this diet. To resolve this question eight students were placed on their normal diet plus one gram of ascorbic acid per day for a period of three days. A 24 hour urine specimen was collected on the third day of this period and analyzed for glucuronic acid. The data obtained in

this experiment (Table II) showed no significant increase in glucuronide excretion as compared with values obtained for these individuals on their normal diet not supplemented with vitamin C.

DISCUSSION.—On the basis of the data obtained in these experiments, it is evident that the acid or alkali in the diet, of themselves, have very little control over the urinary excretion of glucuronic acid. For this reason, it is likely that a different mechanism is responsible for the decreased glucuronide excretion observed by Tseng, *et al.* (1950) when salicylates plus sodium bicarbonate were administed to humans. These investigators also reported an increased salicylate excretion when bicarbonate was administered along with salicylates. For these reasons, it may be postulated that the administration of alkali along with salicylate enhances the excretion of salicylates by the kidney thus leaving less circulating salicylate to be detoxicated in the body. These findings indicate the need for continued dietary management of calculus patients in order to maintain an acid urine if the salicylate therapy suggested by Prien and Walker (1955, 1956) is to have its maximal effect.

The observed decrease in glucuronide excretion by those subjects on a short term fast is probably associated with the fact that the intake of substances in the diet, normally excreted as glucuronides, was abolished during the fast. The two- to three-fold increase in glucuronides by subjects on the citrus fruit diet remains unexplained. Since incorporation of ascorbic acid into the diet at the level of 1 gm. per day failed to increase the glucuronide excretion, the observed increase on the citrus fruit diet could not have been associated with its vitamin C content. The failure of vitamin C to enhance the excretion of glucuronic acid confirms the earlier work of Tseng, *et al.* (1950).

SUMMARY

Glucuronic acid excretion has been studied in humans on several dietary regimens. It does not appear to be affected by the acid or alkali content of the diet *per se*. The inclusion of large quantities of citrus fruit in the diet causes a two- to three-fold increase in glucuronide excretion. This increase is not associated with the vitamin C content of the diet.

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IMMUNITY TO *TRICHINELLA SPIRALIS* IN MICE INFECTED WITH IRRADIATED LARVAE[1]

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The effect of irradiation on *Trichinella* has been studied by many investigators following the report of Schwartz (1921) that x-rays exerted a more or less selective action on the gonads of the parasite so that normal development in the intestine of the suitable host was not possible (Semrad, 1937; Evans, Levin, and Sulkin, 1941; Levin and Evans, 1942; Alicata and Burr, 1949). Levin and Evans (*loc. cit.*) showed that the intestinal infection alone was able to produce immunity to a second infection. They irradiated the larvae before infection with 3,250 r or 3,750 r to prevent sexual maturity of the developing worms. Gould, Van Dyke, and Gomberg (1953) found that dosages from 5,000 r to 6,000 r prevented the larvae from maturing into adult worms.

In view of these findings, it seemed worthwhile to use x-ray as a means of eliminating one or more phases of the life cycle to determine the relative importance of the various phases in producing the total immunity. For this consideration, the life cycle of *T. spiralis* was divided arbitrarily into three phases: phase I—the pre-adults, phase II—the adults, and phase III—the larvae released from the females until encystment in the musculature.

Materials and Methods

The white mice and the strain of *T. spiralis* used in this study were those that have been maintained in the Department of Parasitology for many years. The procedures for the isolation, standardization, and administration of infective larvae, and the procedures for the isolation and counting of adults and larvae were essentially the same as those described previously (Larsh and Kent, 1949; Larsh *et al.*, 1953), with minor changes, such as the dose of infecting larvae. The procedures used to recover and count pre-adults were the same as those for adult worms.

The protocol for the irradiation of larvae was as follows. They were suspended in 15 cc. or 30 cc. of nutrient broth with 2 per cent gelatin, and poured into a pyrex Petri dish. The Petri dish, without its cover, was placed under the source of x-rays at a distance of 18 cm. and the contents were irradiated at the desired dosage. The source of radiation was a Picker 220 KV Half Wave Therapy Machine set at 220 kv., 20 ma., with 0.5 mm. *Cu* and 1 mm. *Al* filtration. The

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half value layer was 1.05 mm. of *Cu*. The dose rate ranged from 576 r to 620 r per minute in air as measured with a Victoreen condenser r-meter. The irradiated larvae were then pipetted into a clean, warm 30 cc. conical centrifuge tube containing nutrient broth with 5 per cent gelatin. The procedures for standardization of, and infecting with, the irradiated larvae were identical with those for the non-irradiated larvae.

In Experiments II-V, following the adult worm counts on the 7th day after the challenging infection, the worms were preserved in 10 per cent formalin for later measurements of females. Twenty-five female worms were selected from each group at random and were measured one at a time by means of a microscope placed beneath a photographic enlarger, which was converted into a copying device. The image from the stage of the microscope was projected onto the viewing glass plate on top of the converted photographic enlarger, and the outlines of the worms were drawn on a piece of onion skin paper. The length of the female worms was measured with a map measuring instrument. The actual length of the worms was calculated by means of a conversion factor (0.052), which was obtained by calibrating the map measuring instrument in reference to a stage micrometer.

The following criteria were used to determine the presence of immunity: (1) the number of adult worms present on the 7th day following the challenging infection, (2) the length of 25 female worms collected on the same day, and (3) the number of larvae present on the 28th day following the challenging infection.

Student's "t" test was used to determine the statistical significance of the observed differences in the adult worm counts, female worm measurements, and larvae counts in each experiment. The statistical significance of the observed differences between groups from different experiments was analyzed by the analysis of variance test. A value of 0.05 or less was accepted as significant.

EXPERIMENTAL PROCEDURES AND RESULTS

In using irradiation as a means of studying the contribution of the various phases of *T. spiralis* to the total immunity produced by mice, it was first necessary to determine the effective dosages of irradiation that will: (1) sterilize the adult worms, and (2) prevent maturation of the larvae into adult worms. To make these determinations, several dosages were tested in a series of preliminary experiments. The experimental mice received the irradiated larvae and the controls received the same number of non-irradiated larvae. Adult worm counts were made at intervals after the second day to compare the percentage of establishment and the longevity of the worms in the two groups.

The absence of muscle larvae 28 to 35 days following a single infection was used as the criterion for the sterilization of the adults (i.e., the elimination of phase III). The morphologic characters of the worms on the third day after infection were used to determine whether the infecting larvae had or had not developed into adults (i.e., remained in phase I, the pre-adult phase).

EXPERIMENT I. *Purpose.*—A composite experiment was designed to verify the preliminary findings of the effects of the various dosages of irradiation upon

TABLE I

The average number of pre-adults (phase I) and adults (phase II) of T. spiralis *recovered from the small intestine of mice following a single infection with 200 non-irradiated larvae or 200 irradiated larvae*

Days Following Infection	Larvae Not Irradiated (Control)			Larvae Irradiated with 1,750 r			Larvae Irradiated with 3,500 r			Larvae Irradiated with 5,250 r			Larvae Irradiated with 7,000 r			Larvae Irradiated with 8,750 r		
	No. mice	Avg. no. adults	Per cent	No. mice	Avg. no. adults	Per cent	No. mice	Avg. no. adults	Per cent	No. mice	Avg. no. pre-adults	Per cent	No. mice	Avg. no. pre-adults	Per cent	No. mice	Avg. no. pre-adults	Per cent
2	6	93.3	46.7	6	163.0	81.5	6	123.5	61.8	6	146.3	73.2	6	35.5	17.8	6	1.0	0.5
7	6	153.7	76.9	6	183.7	91.9	6	192.7	96.4	6	113.6	56.8	6	14.5	7.3	6	0	0
14	6	99.8	49.9	6	137.5	68.8	6	28.2	14.1	6	22.7	11.4	6	2.3	1.2	6	0	0
21	6	42.5	21.3	6	4.3	2.2	6	1.0	0.5	6	4.8	2.4	6	0	0	6	0	0
28	6	11.8	5.9	6	4.3	2.2	6	0	0	6	0	0	6	0	0	6	0	0

establishment of worms after a single infection, and especially to obtain data on persistency, an important condition that was not studied sufficiently in the preliminary experiments.

Procedures.—One hundred and eighty mice, between two and one-half and three months of age, were matched according to sex and separated randomly into six groups. Five groups of mice, 30 mice in each group, were infected with 200 larvae irradiated with 1,750 r, 3,500 r, 5,250 r, 7,000 r, or 8,750 r. A control group of 30 mice was infected with 200 non-irradiated larvae.

At 2, 7, 14, 21, and 28 days following the single infection, worm counts were made from each of the six groups (Table I).

Results.—In general, the establishment and persistency of the worms decreased with increased doses of irradiation. The number of worms in the group that received non-irradiated larvae and in those that received larvae irradiated with 1,750 r and 3,500 r was similar through the seventh day, after which, in general, the reduction in the irradiated groups was greater. In those given larvae irradiated with 5,250 r, the counts were also fairly high through the seventh day, but the decline thereafter was rapid. The highest dosages of irradiation (7,000 r and 8,750 r) resulted in the smallest recoveries and the worms were lost more rapidly than from the other groups.

Observations at various periods after the third day of infection showed that the larvae irradiated with 3,500 r had matured into adult worms. Negative larvae counts on the 28th day following the infection proved that the worms had been sterilized. Therefore, this dosage was chosen as the effective dosage that could be utilized in the following experiments to eliminate phase III.

From morphologic observations of the worms at the time of counting, it was not difficult to determine the irradiation dosages (5,250 r, 7,000 r, and 8,750 r) that prevented the larvae from maturing into adult forms. Mice infected with larvae irradiated with 5,250 r showed that they had not developed beyond phase I. This, in addition to the fact that these irradiated larvae established in num-

bers similar to those for the non-irradiated larvae during phase I (the first 2 days) fulfilled the requirements for this dosage for use in the following experiments to eliminate phases II and III. Although the 7,000 r dosage did not meet entirely the necessary conditions, it seemed permissible to include this dosage in attempts to immunize mice. However, the 8,750 r dosage was eliminated because of the poor recovery even on the second day following infection.

EXPERIMENT II. *Purpose.*—This experiment was designed to determine the degree of immunity produced by non-irradiated larvae (phases I, II, III) and larvae irradiated with 1,750 r (probably all three phases).

Procedures.—Eighteen mice, between the ages of two and one-half and three months, were matched according to sex and separated randomly into three groups. Six mice in Group I were given stimulating infections with larvae irradiated with 1,750 r. The same number of mice in Group II was stimulated with non-irradiated larvae, while the six mice placed in Group III were not stimulated. The mice in Groups I and II were given three stimulating infections of 200 larvae each at 21-day intervals. Two days after each infection, worm recoveries from 4 additional mice (not infected previously) were those expected from a 200 dose of larvae, proving the viability of the larvae used to infect the mice of Groups I and II. Twenty-eight days following the last stimulating infection, all of the mice in Groups I and II were challenged with 200 non-irradiated larvae each. The six mice in Group III that had received no stimulating infections also were given the challenging infection to show the degree of natural immunity in mice of this age.

At seven days following the challenging infection, all of the mice of the three groups were sacrificed for adult worm counts. Also, at this time, length measurements were made of 25 female worms recovered from each group of mice (Table II).

Results.—The data of Experiment II were analyzed by Student's "t" test.

TABLE II

The average number and percentage of T. spiralis *adults and the average length of 25 female worms recovered from experimental and control mice seven days following a challenging infection with 200 non-irradiated larvae*

Irradiation dosage	GROUP I Immunized with irradiated larvae			GROUP II Immunized with non-irradiated larvae			GROUP III Non-immunized		
	No. mice	Avg. no. adults (per cent)	Avg. length females	No. mice	Avg. no. adults (per cent)	Avg. length females	No. mice	Avg. no. adults (per cent)	Avg. length females
1,750 r	6	80.8 (40.4)	2.25 mm.	6	29.7 (14.9)	2.04 mm.	6	131.5 (65.8)	2.70 mm.
3,500 r	6	98.5 (49.3)	2.27 mm.	6	106.7 (53.4)	2.19 mm.	6	155.3 (77.7)	2.80 mm.
5,250 r	6	86.5 (43.3)	2.07 mm.	6	60.5 (30.3)	2.01 mm.	6	106.3 (53.2)	2.89 mm.
7,000 r	6	125.8 (62.9)	2.34 mm.	6	22.2 (11.1)	2.09 mm.	6	139.3 (69.7)	2.89 mm.

The analyses of the adult worm counts showed that there was a significant difference between the count for the non-immunized group (131.5) and that for the group immunized with non-irradiated larvae (29.7). However, there was no significant difference between the non-immunized group (131.5) and the group immunized with larvae irradiated with 1,750 r (80.8). There also was no significant difference between the two immunized groups. It should be added that all of the worms recovered from the Group I mice were sexually mature adults, proving that the 1,750 r dosage had not prevented the maturation of the larvae into adult males and females.

The analyses of the length measurements of female worms showed that there were highly significant differences between the sizes of the worms from the non-immunized group (2.70 mm.) and the immunized groups (2.25 and 2.04 mm., respectively, for Groups I and II). There was also a significant difference between the sizes of the worms from the group immunized with non-irradiated larvae (2.04 mm.) and those from the group immunized with larvae irradiated with 1,750 r (2.25 mm.).

EXPERIMENT III. *Purpose.*—This experiment was designed to determine the degree of immunity produced by non-irradiated larvae (phases I, II, III) and larvae irradiated with 3,500 r (phases I, II).

Procedures.—Thirty mice, between the ages of two and one-half and three months, were matched according to sex and separated randomly into three groups as in the previous experiment. Twelve mice in Group I were stimulated with larvae irradiated with 3,500 r, and nine mice in Group II were stimulated with non-irradiated larvae. All of the mice in these two groups were given three stimulating infections of 200 larvae each at 21-day intervals. In all cases, the larvae used for the stimulating infections proved to be viable as shown by worm recoveries from four additional mice sacrificed two days after each infection. Twenty-eight days following the last stimulating infection, all of the mice in the two groups, and nine mice in Group III that had received no stimulating infections, were challenged with 200 non-irradiated larvae each.

Seven days following the challenging infection, six mice from each of the three groups were sacrificed for adult worm counts, and length measurements were made of 25 female worms recovered from each group (Table II). The remaining six mice in Group I and the remaining three mice in Groups II and III were sacrificed 28 days following the challenging infection and the number of larvae in the musculature of each was determined (see below).

Results.—The data of Experiment III were analyzed by Student's "t" test. The analyses of the adult worm counts showed that there were significant differences between the counts for the non-immunized group (155.3) and the immunized groups (98.5 and 106.7, respectively, for Groups I and II). As expected, there was no significant difference between the counts for the group immunized with non-irradiated larvae (106.7) and the group immunized with larvae irradiated with 3,500 r (98.5).

The analyses of the length measurements of the female worms showed that there were highly significant differences between the sizes of the worms from the non-immunized group (2.80 mm.) and the immunized groups (2.27 and

2.19 mm., respectively, for Groups I and II). As expected, there was no significant difference between the sizes of the worms from the mice immunized with non-irradiated larvae (2.19 mm.) and those from the mice immunized with larvae irradiated with 3,500 r (2.27 mm.).

The analyses of the larvae counts showed that there was a significant difference between the count for the non-immunized group (49,089) and that for the group immunized with larvae irradiated with 3,500 r (14,193). A direct comparison cannot be made with the Group II mice (85,178), since they received non-irradiated larvae in the stimulating infections. Non-irradiated larvae, unlike those irradiated with 3,500 r, develop into sexually mature adults and each female is capable of releasing about 1,500 larvae. This means that after each of the three stimulating infections and after the challenging infection, the females were releasing larvae. On the other hand, due to the effects of irradiation, the release of larvae into the circulation of the mice of Group I occurred only after the challenging infection, which was given with non-irradiated larvae.

EXPERIMENT IV. *Purpose.*—This experiment was designed to determine the degree of immunity produced by non-irradiated larvae (phases I, II, III) and larvae irradiated with 5,250 r (only phase I).

Procedures.—The same number of mice (30) was used and the same procedures were followed in this experiment as in Experiment III, except that in the present case the Group I mice were stimulated with larvae irradiated with 5,250 r.

Results.—The average numbers of adult worms and the average length of 25 female worms recovered seven days following the challenging infection are shown in Table II. The average numbers of larvae recovered 28 days after the challenging infection are listed below.

The data of Experiment IV were analyzed by Student's "t" test. The analyses of the adult worm counts showed that there was a significant difference between the count for the non-immunized group (106.3) and that for the group immunized with non-irradiated larvae (60.5). However, there was no significant difference between the count for the non-immunized group (106.3) and that for the group immunized with larvae irradiated with 5,250 r (86.5). There also was no significant difference between the counts for the two immunized groups (86.5 and 60.5, respectively, for Groups I and II).

The analyses of the length measurements of female worms showed that the differences in sizes between those from the non-immunized group (2.89 mm.) and those from the immunized groups (2.07 and 2.01 mm., respectively, for Groups I and II) were highly significant. However, there was no significant difference between the sizes of the worms from the group immunized with non-irradiated larvae (2.01 mm.) and from the group immunized with larvae irradiated with 5,250 r (2.07 mm.).

The analyses of the larvae counts showed that there was a significant difference between the count for the non-immunized group (35,733) and that for the group immunized with larvae irradiated with 5,250 r (8,535). A direct comparison cannot be made with the Group II mice (78,818) for reasons mentioned earlier.

EXPERIMENT V. *Purpose.*—This experiment was designed to determine the

degree of immunity produced by non-irradiated larvae (phases I, II, III) and larvae irradiated with 7,000 r (only phase I).

Procedures.—The same number of mice (18) and the same procedures were followed in this experiment as in Experiment II, except that in the present case the Group I mice were stimulated with larvae irradiated with 7,000 r.

Results.—The average numbers of adult worms and the average length of 25 female worms recovered seven days following the challenging infection are shown in Table II.

The data of Experiment V were analyzed by Student's "t" test. The analyses of the adult worm counts showed that there was a significant difference between the count for the non-immunized group (139.3) and that for the group immunized with non-irradiated larvae (22.2). However, there was no significant difference between the count for the non-immunized group (139.3) and that for the group immunized with larvae irradiated with 7,000 r (125.8). As expected, there was a significant difference between the two immunized groups (125.8 and 22.2, respectively, for Groups I and II).

The analyses of the length measurements of female worms showed that there were significant differences in the sizes of the worms from the non-immunized group (2.89 mm.) and from the immunized groups (2.34 and 2.09 mm., respectively, for Groups I and II). There also was a significant difference in the sizes of the worms from the two immunized groups.

ANALYSES OF EXPERIMENTS II, III, IV, AND V.—The analysis of variance test was performed on the results of the adult counts obtained from Groups I (1,750 r, 3,500 r, 5,250 r, and 7,000 r) of Experiments II, III, IV, and V. The analyses were based on the mean values of each group. There were no significant differences between the means of Groups I, except between the means of 1,750 r and 7,000 r, the former being significantly smaller than the latter.

The analysis of variance test was performed on the length of the female worms recovered from Groups I (1,750 r, 3,500 r, 5,250 r, and 7,000 r) of Experiments II, III, IV, and V. There were no significant differences between the following dosages: 1,750 r, 3,500 r, and 7,000 r. However, the mean of the 5,250 r group was significantly smaller than the others.

The observed differences in the larvae counts of Experiments III and IV show that in both experiments the non-immunized mice (Group III) harbored considerably greater numbers of larvae than the mice immunized with irradiated larvae (Group I). In fact, the counts are more than three times greater in the non-immunized mice.

DISCUSSION

The results of Experiments II–V showed that the mice stimulated with non-irradiated larvae or larvae irradiated with 3,500 r had developed acquired immunity, as measured by adult worm counts made at seven days after the challenging infection. These results agree with those of Hendricks (1952) obtained following the use of a similar irradiation dosage. The mice stimulated with larvae irradiated with 1,750 r harbored considerably fewer adult worms than

their non-immunized controls, but the observed difference in the counts is not significant statistically. Those that were stimulated with larvae irradiated with 5,250 r or 7,000 r did not develop a demonstrable degree of immunity as measured in this way. These results suggest that phase I alone (5,250 r or 7,000 r) is not as effective as phases I and II combined (3,500 r) in producing an immunity that causes a significant expulsion of adult worms within seven days after a challenging infection. In this connection, there is no explanation for the failure of the 1,750 r dosage (probably all three phases) to produce a significant reduction in worms within this period. However, the great reduction noted suggests that some immunity was developed (Table II). This is also suggested by the fact that the counts for the mice stimulated with this dosage and those stimulated with non-irradiated larvae are not different statistically. Among the stimulated groups, there is no statistical difference in the adult worm counts of those immunized with non-irradiated larvae and those immunized with larvae irradiated with 1,750 r, 3,500 r, or 5,250 r. Thus, despite the fact that the 1,750 r and 5,250 r dosages failed to produce a demonstrable immunity, this fact suggests that some immunity had been developed but was too feeble to be shown in this way. If so, then phase I, the only phase common to the non-irradiated larvae and those treated with 1,750 r, 3,500 r, and 5,250 r, may be considered important in stimulating immunity. The 7,000 r dosage showed no indication of producing immunity, since the counts are significantly greater than those from the mice stimulated with non-irradiated larvae (Table II). However, it should be added that the results with this dosage were anticipated in view of the findings of Experiment I (Table I). Compared with the other irradiation dosages, the 7,000 r treatment resulted in the establishment and maintenance of relatively few worms after one infection. The degree of antigenic stimulation, therefore, would be expected to be considerably less following infection with larvae treated with 7,000 r than with larvae treated with the other irradiation dosages.

The fact that there was no significant difference between those immunized with non-irradiated larvae (phases I, II, III) and those immunized with larvae irradiated with 3,500 r (phases I, II) suggests that phase III is not likely to be involved to a demonstrable degree in the production of immunity. This agrees with reports that antibodies formed against phase III larvae are non-protective in action (Oliver-Gonzalez, 1941). Nevertheless, definite conclusions probably will not be possible until means are discovered to study the effects on this immunity of phase III alone.

The fact that all of the immunized groups of mice in Experiments II–V had female worms significantly shorter than those in the non-immunized mice is proof that immunity had developed as a result of the various stimulating infections. Thus, it is clear that this criterion is a more delicate measure of immunity than adult worm counts at seven days after a challenging infection. It is interesting that the size of the stunted females did not differ significantly in the mice immunized with non-irradiated larvae and in those immunized with larvae irradiated with 3,500 r (phases I, II) or 5,250 r (phase I). The fact that the shortest worms were recovered from the mice immunized with larvae ir-

radiated with 5,250 r suggests that phase I is the most important in this respect. It is possible that the inhibitory effects are most detrimental during phase I (the first two days) thus making normal development impossible. If so, the mice with larvae irradiated with 5,250 r (phase I) would be expected for two main reasons to show the strongest effect against the developing worms. In the first place, the immunologic responses against a single phase may be stronger than against the same phase when it occurs as one of a combination of phases. Of greater importance is the fact, emphasized in the preliminary experiments, that larvae irradiated with 5,250 r (phase I only) persist for long periods after infection, whereas phase I under usual conditions (non-irradiated larvae, larvae irradiated with 1,750 r or 3,500 r) lasts only about two days. Further work is needed, however, before definite conclusions can be drawn. The fact that the 7,000 r dosage (phase I) did not produce the same degree of stunting as the 5,250 r dosage probably was due, as explained above, to the much smaller number of worms that persisted after the stimulating infections.

The results of the larvae counts showed that the mice immunized with irradiated larvae (3,500 r or 5,250 r) developed a striking acquired immunity. The fact that larvae counts are estimates and the animals are limited in number, the results from Groups I of the two experiments did not warrant further statistical analysis beyond that mentioned previously for each experiment. However, it is clear that in both of the irradiation groups there was produced an immune response that interfered with the growth (as revealed by length measurements) and reproductive potential of the female worms.

On the basis of these various results, it would seem safe to conclude that phase I is an important contributor to the total immunity of the host. Thus, it appears that it plays a much more important role in the immunity to *T. spiralis* than believed formerly. Since this phase is the first to enter into combat with the host, a thorough understanding of its role in the immunity may provide the information necessary to work out the details of the mechanism of the immunity. In consideration of this, further research on phase I is encouraged.

SUMMARY

The larvae of *T. spiralis* were irradiated with certain dosages (1,750 r, 3,500 r, 5,250 r, or 7,000 r) to eliminate one or more phases of the life cycle. These irradiated larvae were used in giving three stimulating infections and the degree of immunity produced was measured by adult worm counts and length measurements of female worms seven days following a challenging infection with non-irradiated larvae, and by larvae counts twenty-eight days following this infection. The degree of immunity demonstrated was compared with that produced in mice given the same number of stimulating infections with non-irradiated larvae. By using one or more of the three criteria of immunity, it was shown that all of the groups given the stimulating infections had developed immunity. While the results seem to preclude the migrating and encysting larvae (phase III) as contributors to the total immunity, it is clear that both the pre-adults (phase I) and adults (phase II) play important roles, and that the pre-adults are more important in this connection than believed formerly.

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LARVAL DEVELOPMENT OF THE CRUSTACEAN *THOR FLORIDANUS* KINGSLEY[1, 2]

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Three tiny, ovigerous, female shrimp were found in August, 1954, among sea squirts and coral near the shore of the Duke University Marine Laboratory at Beaufort, N. C. These individuals were placed in separate bowls of sea water in the laboratory and held until the eggs hatched. The adults were then preserved

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and later identified as *Thor floridanus* by Dr. Fenner A. Chace, Jr. This species has not since been encountered at Beaufort.

The larvae were reared through metamorphosis in the laboratory. These larvae, while resembling those obtained from Bermuda plankton and assigned to the genus *Thor* by Lebour (1940), differed from Lebour's material in size and color and from her reconstructed developmental sequence in both number and structure of intermolts. Since the present larvae are undoubtedly those of *T. floridanus*, the species to which inference assigns Lebour's material, two alternatives are suggested: the two groups of larvae belong to different taxonomic groups; or the larvae belong to the same species but their development varies in accordance with geographic location or other external factors.

The author wishes to acknowledge with thanks his indebtedness to Professor C. G. Bookhout, under whose guidance and direction this work was done. Thanks are also due Dr. T. R. Rice for cultures of algal cells fed to larvae, and Dr. Chace for identification of adults.

METHODS.—Ten larvae from the same clutch of eggs were placed in each of several clean finger bowls of untreated sea water. The larvae were fed clumps of cells from uni-algal cultures made from T. R. Rice's pure stocks of *Chlamydomonas* sp., *Thorocomonas* sp., *Nannochloris* sp. (Chlorophyta), or the diatom, *Nitzschia closterium*. Each individual received the same food throughout its life.

Each larva and bowl was examined daily under a dissecting binocular microscope. Only the presence of all or parts of exuviae was accepted as evidence of ecdysis. Water was changed and food added after each inspection.

Living larvae, anesthetized in a dilute urethane-sea-water solution, were drawn with the aid of a camera lucida. Individual appendages were studied from alcoholic specimens which had been anesthetized, killed in formalin and partially dehydrated in a graded series of alcohols to prevent distortion of the exoskeleton.

RESULTS.—The three females produced respectively 108, 125 and 207 living first zoea larvae. The eggs hatched at night and the parent shrimp molted shortly afterward. One of the three females produced a second clutch of eggs immediately following the post-incubatory ecdysis.

The larvae usually survived the first molt whether they had been fed or not. Twenty-three individuals, out of a total of 30 which were not fed, molted once. None of these molted twice and all died within five days. Of 20 larvae fed *Chlamydomonas*, 12 molted once, none molted twice, nor did any live longer than five days. Forty larvae were fed *Thorocomonas*. Although none of these metamorphosed, five individuals completed the larval phase of development. Twenty first zoeae were fed *Nannochloris*. All of these survived the first molt, and seven lived through metamorphosis. Survival among 40 larvae fed *Nitzschia* was influenced by the removal of specimens for preservation and study. Thirty individuals lived through one molt, and two were left after metamorphosis.

The first molt occurred on the second or third day after hatching. The frequency of subsequent ecdyses among those larvae which survived was one molt every 1.9 to 2.0 days. The final larval form was attained after the seventh molt. Metamorphosis occurred at the eighth ecdysis.

First zoea (Fig. 1–5): length 1.6 mm. Carapace with pterygostomian spine; antero-ventral margin of carapace behind this spine with 3 serrations or teeth. No true rostrum, the carapace terminating anteriorly in a broad flat point between the sessile eyes. Dorsal organ in mid-dorsal line above posterior margin of orbit. Dorsal papilla in mid-dorsal line near posterior margin of carapace. Abdomen of 6 somites, without spines. Abdominal somite 6 continuous with telson. Telson roughly triangular with terminal corners truncated and posterior margin notched medially; bearing 14 terminal spines.

Antennule (Fig. 2) simple with a long basal segment which bears distally a long plumose seta on the inner side and an outer flagellum of 1 segment; outer flagellum with 3 club-like aesthetes and a short plumose seta. Antenna biramous; basal segment with a tooth at junction with flagellum; flagellum of 1 short segment with a long plumose seta and a short spine at its distal end; scale consisting of a long proximal and 4 short distal segments with 2 setae externally and 9 long plumose setae around the tip and inside.

Mandible (Fig. 3) with short, 3-toothed incisor process and a toothed molar surface; two small serrate teeth in angle between incisor and molar processes. First maxilla (Fig. 4) with coxa armed with 2 stout teeth and 3 spines; basis with 6 serrate teeth; endopod a 2-jointed palp with 3 setae with stout side bristles and a sparcely bristled terminal spine. Second maxilla (Fig. 5) biramous, the basis 3-lobed, bearing stout spines and bristled setae on the lobes; endopod bearing 3 bristled setae on a lobe near its base, 2 bristled setae on a median lobe, one bristled seta on a lobe near the apex, and 2 bristled apical setae; exopod a flat plate with 3 anterior and one lateral plumose setae.

First maxilliped biramous; basis with 3 inwardly directed setae; endopod of 4 segments each of which bears 1 or 2 setae distally; exopod longer than endopod with 4 apical and 2 sub-apical setae. Second maxilliped similar to first but larger; endopod with 3 apical spines; exopod with 3 apical and 2 sub-apical setae. Third maxilliped biramous; endopod of 4 segments, unlike other maxillipeds, with terminal setae on ultimate and penultimate segments; exopod with 3 apical and 2 sub-apical setae.

First pereiopod biramous, rudimentary, small.

Bright red, stellate chromatophores at distal and proximal ends of antennular peduncle, on labrum, in mid-dorsal line above gastric and cardiac regions, in mid-dorsal line of each abdominal somite, on sternite of thoracic somite 2, on sternite of abdominal somites 3, 4 and 6, anterior to and on each side of anus, and 1 on each side near end of telson. Larvae otherwise colorless.

The first zoea corresponds closely to Lebour's description of the first larval stage. Lebour's larva was 2 mm. or less in length as opposed to 1.6 mm. for the present first larva. Lebour's figure of the first maxilla shows an unsegmented endopod and setation which differs slightly from that of my material in which the enopod is of 2 segments. Setation of the second maxilla differs between Lebour's larvae and the first zoea of *T. floridanus*. Differences in color pattern which apply to all larvae are treated elsewhere.

Second zoea (Fig. 6): length 1.9 mm. Eyes stalked; eyestalks with postero-

ventral, red chromatophores. Rostrum flat, broadly triangular, not extending beyond eyes. Carapace with spines and dorsal papillae of previous zoea plus an antennal spine. Telson as before, but with 2 minute terminal spines in the midline.

Antennular peduncle of 2 segments, the proximal segment being over 3 times as long as the distal and bearing 2 setae; distal segment with an inner distal seta borne on a short, bulbous base, and a seta antero-laterally; outer flagellum as before, but the 3 aesthetes are now long. Antennal scale of a long proximal and 3 short distal segments; flagellum without long terminal seta, now ending in a short spine.

Appendages otherwise as before, except as noted. Exopod of first maxilliped with 5 terminal setae. Endopods of second and third maxillipeds now of 5 segments. Rudimentary first pereiopod now quite large. Second pereiopod tiny, biramous, rudimentary.

The second zoea of *T. floridanus* corresponds to Lebour's description of the second larval stage except that Lebour's larva lacks the rudiments of the second pereiopods.

Third zoea (Fig. 7): length 2.0 mm. Telson separate from 6th abdominal somite, no wider posteriorly than anteriorly, with lateral spines about ⅔ the distance from the proximal end and 2 large and 2 minute terminal spines.

Base of antennular peduncle expanded laterally (stylocerite); basal segment with 2 setae on the inside and a distal band of short setae dorso-laterally; ultimate segment with 2 ventro-distal setae, a minute inner flagellum bearing a long seta, an antennular lobe, and a short outer flagellum bearing 2 aesthetes. Antennal scale consisting of a basal and 2 distal segments with 10 long plumose setae.

Other appendages essentially as in second zoea with the following exceptions; Exopod of second maxilla with 4 anterior and one posterior plumose setae. First pereiopod biramous; endopod very short, of 5 segments; exopod long with 3 apical and 2 sub-apical setae. Second pereiopod biramous, rudimentary, large.

Uropod biramous, unsegmented; outer ramus with 6 setae, inner ramus shorter than outer, with 2 minute setae.

The third zoea probably differs from Lebour's third stage in size and development, although she reports considerable variation in regard to both. Lebour's third stage is 2.8 mm. in length as opposed to 2.0 mm. for the third zoea of *T. floridanus*. Lebour's third stage has more pereiopod rudiments and, in larger individuals, suggestions of pleopods. It is generally more advanced than my third zoea.

Fourth zoea. (Fig. 8, 9): length 2.0 mm. Differs only slightly from 3rd zoea. Telson with terminal spines of each side of center notch arranged from lateral to medial: 2 minute spines, 1 large spine, 1 small spine, 1 large spine, and 1 small spine.

Antennular peduncle with longer basal segment imperfectly divided into a long proximal and a short distal segment; lines of separation of peduncle into segments marked by an inner seta and a band of short setae dorso-laterally; ultimate segment of peduncle with 4 setae ventrally at distal end; inner flagellum

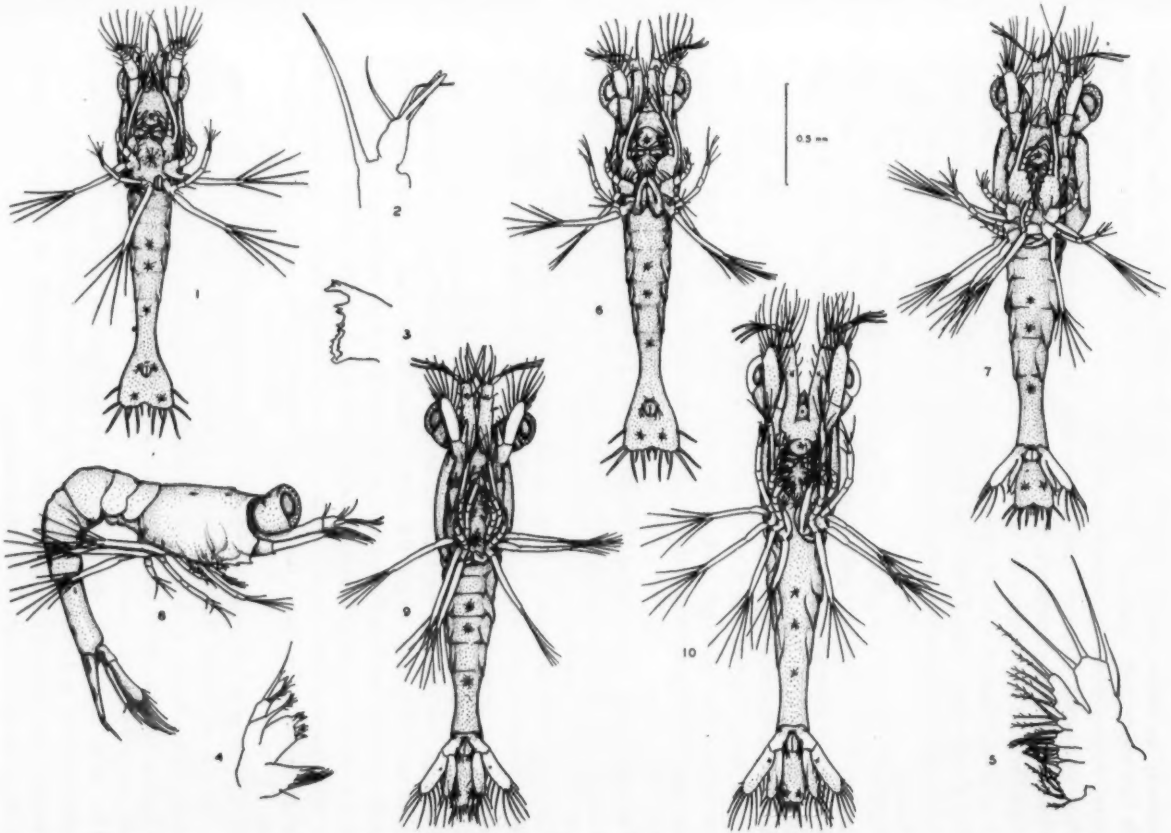

FIG. 1–10. *Thor floridanus*. FIG. 1, 6–10, ×30. FIG. 2–5, ×130. Setules omitted from plumose setae. FIG. 1–5. First zoea. FIG. 1. Entire larva, ventral view. FIG. 2. Tip of antennule. FIG. 3. Mandible. FIG. 4. First maxilla. FIG. 5. Second maxilla. FIG. 6. Second zoea, ventral view. FIG. 7. Third zoea, ventral view. FIG. 8, 9. Fourth zoea. FIG. 8. Lateral view. FIG. 9. Ventral view. FIG. 10. Fifth zoea, ventral view.

essentially as before; outer flagellum with 2 aesthetes and 2 setae. Antennal scale not segmented, bearing a short spine in antero-lateral corner and 13 long plumose setae around the tip and on inside.

First pereiopod stronger than in previous larva, propodus slightly swollen internally. Uropod segmented, endopod and exopod about equal in length; endopod with 2 short spines on outer edge and 7 plumose setae around the tip and on inside; exopod with 10 setae.

The fourth zoea falls within the limits of variation of Lebour's third stage larva except for a more advanced condition of the telson and uropods in *T. floridanus*.

Fifth zoea (Fig. 10): length 2.1 mm. The fifth zoea differs from the fourth in the following: Telson slightly narrower posteriorly with 3 lateral spines on each side and 2 pairs of alternate long and short terminal spines on each side of the slight median notch.

Antennular peduncle with a short, stout tooth about midway of the long basal segment on the ventral side; 4 setae on inside of peduncle; 5 setae ventro-distally; outer antennular flagellum with 3 aesthetes. Antennal scale with 14 plumose setae; antennal flagellum still short, unsegmented, but now without the terminal spine.

First pereiopod with propodus swollen. Second pereiopod biramous; endopod rudimentary with 2 short apical setae; exopod long with 4 apical and 2 sub-apical setae. Third pereiopod a uniramous rudiment.

Uropodal endopod with a single short spine in posterolateral corner and 8 plumose setae; exopod with a short spine in postero-lateral corner and 10 to 12 plumose setae around the tip and inside.

The fifth zoea, although smaller, bears some resemblance to Lebour's fourth larval stage but yet does not have the last two decapodal legs or pleopods.

Sixth zoea (Fig. 11): length 2.8 mm. Telson longer than previously, tapered slightly, median notch in terminal end very small, spines as before.

Antennule unchanged. Basis of antenna with spines on ventral side, at articulation with flagellum and at articulation with scale; inner ramus separated into a short peduncle and a longer flagellum, not equal to scale; scale with 15 plumose setae.

First pereiopod with propodus forming a small thumb in opposition to dactylus. Second pereiopod with endopod of 5 segments, the propodus with a short thumb. Third, fourth and fifth pereiopods long uniramous rudiments.

First to fifth pleopods minute, uniramous buds. Uropodal endopod with 10 setae; exopod with 13 setae.

The sixth zoea of *T. floridanus* is intermediate in development between Lebour's fourth and fifth stages, but about equal in length to her fourth.

Seventh zoea (Fig. 12): length 3.0 mm. Telson essentially as before but longer and narrower posteriorly. Anal spine stronger.

Inner antennular flagellum longer, but still shorter than outer; outer antennular flagellum with 3 apical aesthetes and a seta and an aesthete which arises midway of the segment. Antennal flagellum of 4 segments, slightly longer than scale.

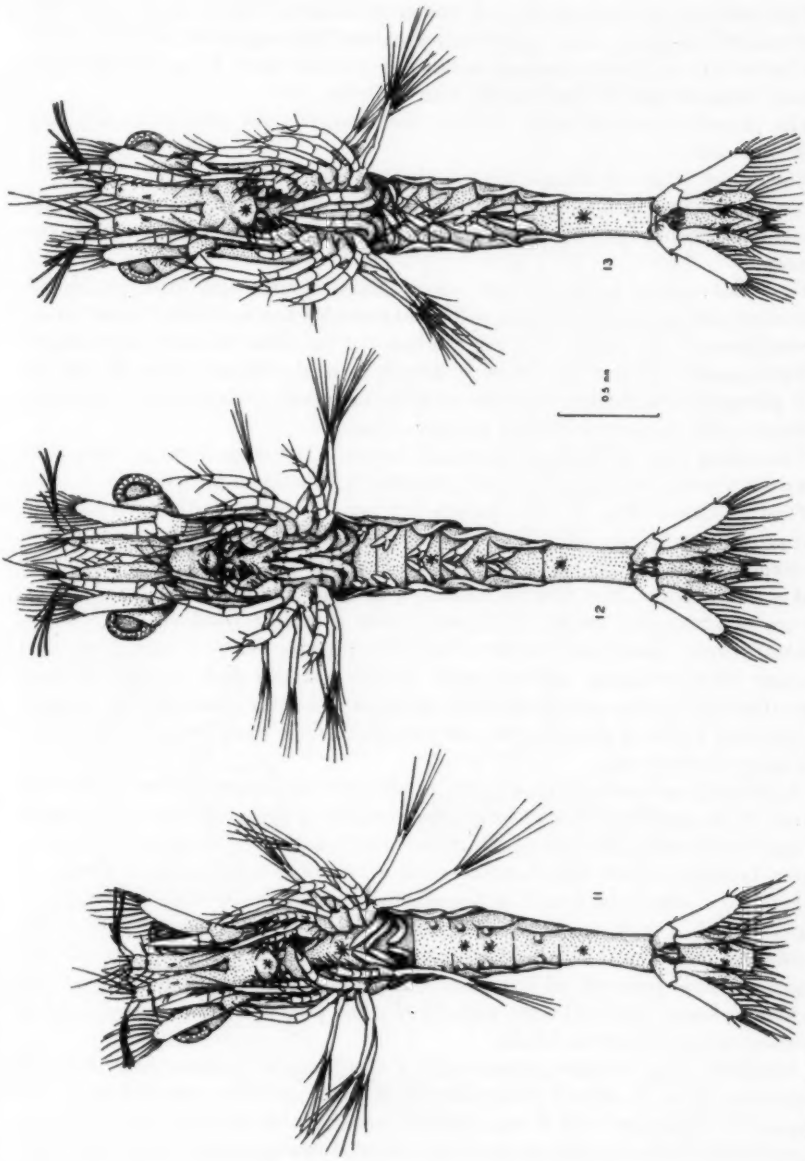

FIG. 11–13. *Thor floridanus*. ×30. FIG. 11. Sixth zoea, ventral view. FIG. 12. Seventh zoea, ventral view. FIG. 13. Eighth zoea, ventral view.

First and second pereiopods with endopods chelate. Third, fourth and fifth pereiopods uniramous, long, imperfectly separated into segments, without setae.

First to fifth pleopods biramous rudiments, without setae. Uropod larger than before, endopod with 11 and exopod with 15 setae.

The seventh zoea, although smaller, corresponds quite closely to Lebour's fifth stage larva.

Eighth zoea (Fig. 13): length 3.0 mm. Form essentially unchanged.

Antennular flagella about equal in length; outer with 3 terminal aesthetes and 3 aesthetes arising from midway of the segment. Antennal flagellum of 5 segments.

First and second pereiopods as before. Third, fourth and fifth pereiopods uniramous, of 4 segments, without setae and probably not functional in the larva; carried beneath the thorax between the bases of the other thoracic appendages.

First pleopod with rudimentary endopod, exopod without setae. Second to fifth pleopods with 2 apical setae on exopods, endopods without setae. Uropodal endopod with 12, exopod with 17 plumose setae.

The eighth zoea, although smaller than Lebour's last stage larva, is somewhat more advanced in having setose and presumably functional pleopods.

First postlarva (Fig. 14, 15): length 3.2 mm. Carapace with antennal and pterygostomian spines, a tooth on the antero-ventral margin posterior to pterygostomian spine, dorsal organ and a dorsal papilla just ahead of the posterior end of the carapace. Rostrum flat, broadly triangular, not extending beyond eyes. Pleura of abdominal somites 4, 5 and 6 with a minute tooth in the postero-ventral corner. Anal spine between bases of uropods. Telson a long, truncated triangle with a minute, median point at the posterior end, 3 pairs of short lateral spines, 2 pairs of long terminal spines of which the outer pair are slightly longer, and a pair of slender setae one of which arises from between the terminal spines on each side.

Antennular peduncle of 3 segments, the basal with a short stylocerite bearing 3 setae in its angle and 2 at its base; segmentation of peduncle marked by bands of short setae dorso-laterally; inner antennular flagellum of 3 segments, equal to outer, bearing 2 short apical setae; outer antennular flagellum imperfectly divided into 4 segments, a tuft of 3 aesthetes at junction of segments, ultimate segment with 3 short apical setae. Antennal basis of 2 obliquely separated segments, the outer basal segment with a ventral spine at the articulation with the scale; antennal peduncle of 3 segments, flagellum of many segments, over half as long as body; antennal scale with tip of spine projecting free of blade and 23 plumose setae around the blade.

Mandible large, incisor process with 4 teeth, molar process many-toothed. First maxilla small, armed with many short teeth on basis, endopod short, unsegmented, palp-like with 1 seta. Second maxilla with 3-lobed base, the lobes armed with 5, 7 and 8 long coarse setae; endopod unsegmented with a long and a short apical seta and a long seta on a lobe midway of the segment; exopod flattened, armed anteriorly with 10 plumose setae and posteriorly with a seta.

First maxilliped biramous with a bilobed epipod; coxa and basis armed

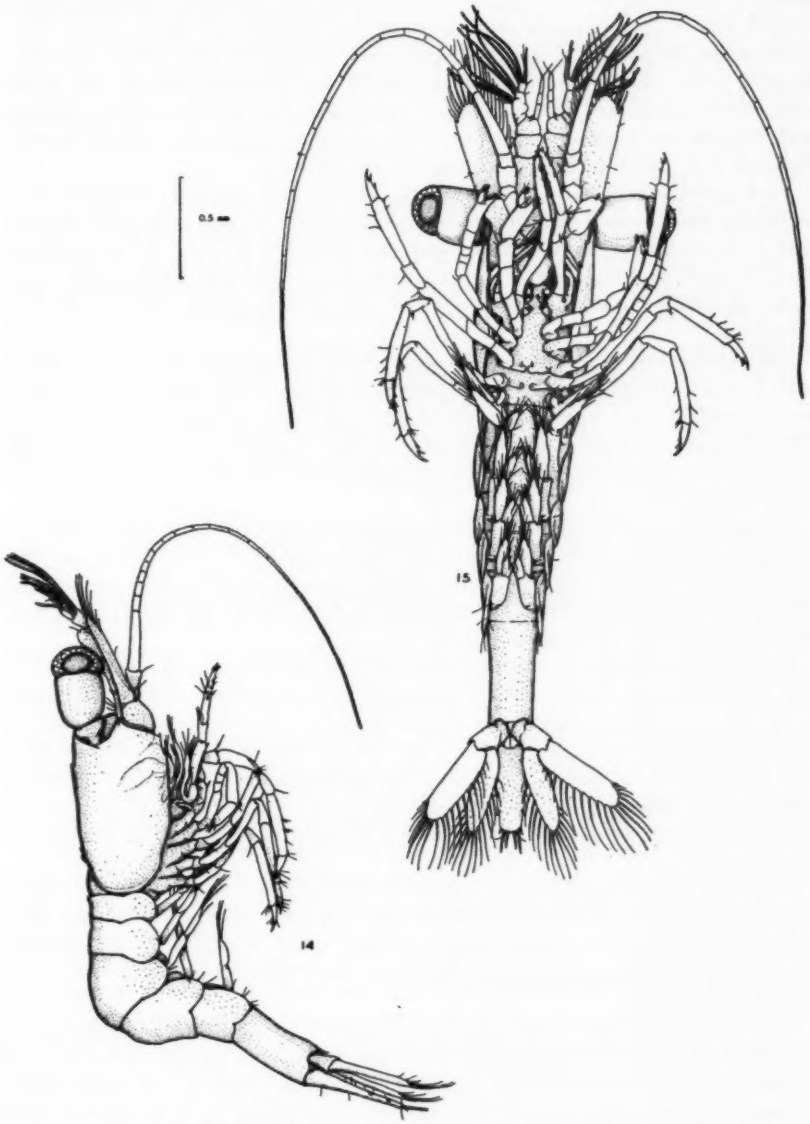

FIG. 14, 15. *Thor floridanus*. ×30. FIG. 14. Postlarva, lateral view. FIG. 15. Postlarva, ventral view.

medially with stout setae or spines; endopod reduced, palp-like, bearing 3 setae; exopod with 3 long plumose setae near proximal end and 4 apical and 2 sub-apical setae. Second maxilliped with coxa and basis armed with stout, inwardly directed setae; endopod of 5 segments, flexed, the segments armed with stout setae which face inward, dactylus with 9 stout setae; exopod longer than endopod, with 4 apical and 1 sub-apical setae. Third maxilliped with rudimentary exopod; endopod of 3 segments, armed with spines and bristles.

First pereiopod chelate, with a short, rudimentary exopod; endopod of 5 segments, dactylus and thumb with bifid, curved tips. Second pereiopod chelate, with a short rudimentary exopod; endopod with carpus of 3 or 4 segments, dactylus and fixed finger bifid at tip. Third, fourth and fifth pereiopods uniramous, dactyls bifid at tip, armed apically with 2 short claws and a short sub-apical spine.

First pleopod with a short, rudimentary endopod armed with 2 setae; exopod with 6 setae. Second and third pleopods biramous, setose, endopods bearing a seta on a slight knob which arises about mid-way along the segment. Fourth and fifth pleopods biramous, setose, endopods with *appendices internae*. Uropodal exopod with a tooth in the postero-lateral corner and 19 plumose setae; endopod with 15 setae.

The postlarva is smaller than Lebour's postlarva, from which it differs slightly. Lebour's postlarva lacks pterygostomian spines and denticles on the antero-ventral margin of the carapace. Lebour found a postero-lateral tooth on only the pleura of abdominal somite 5 as opposed to small teeth on somites 4, 5 and 6 in the postlarva of *T. floridanus*.

DISCUSSION.—Only one generalization may be derived from the rearing of *T. floridanus* larvae in the laboratory: some, but not all, species of unicellular algae constitute an adequate diet for survival, molting and metamorphosis. Neither *Nitzschia closterium* nor *Nannochloris* sp. may be considered an optimum diet. The lack of data for larvae reared on a diet containing animal tissue is a serious one. If more larvae had been available, or if the larvae obtained had not all become available at the same time, more experimental culture work might have been possible.

The inference that Lebour's larvae are *T. floridanus* is based on Verrill's (1922) report of this species at Bermuda. Subsequent attempts to obtain it or other *Thor* species there have been unsuccessful (Lebour, 1940). Holthius (1947) includes Bermuda in the distribution of *T. floridanus*, but it is possible that he also refers to Verrill's report.

A positive suggestion of systematic difference between Beaufort *T. floridanus* and the group to which Lebour's larvae belong is found in the differences in color pattern between Lebour's larvae and those hatched from *T. floridanus* eggs. Lebour's larvae were colored "bright red with many chromatophores on a yellow ground, there being two red streaks from the thorax right along the abdomen." The Beaufort larvae were colorless but bore a single row of stellate red chromatophores on the abdomen. These, however, were not so close together as to suggest a continuous stripe or streak of color. Gurney (1942) reviewed the

importance of pigment patterns in separating larvae of closely related species and concluded that primary chromatophores are specifically constant in number and position. More recently, Broad (1957) has distinguished two species of *Palaemonetes* during the larval phase on the basis of primary chromatophore distribution.

It is difficult to say how much importance may be attached to the differences in size and the number and form of intermolts between Lebour's larvae and those raised in the laboratory. Gurney (1942) feels that the mode of development is constant for every species. Under laboratory conditions, however, variation in the number, size and structure of larval intermolts, caused by diet, salinity or temperature, has been reported for *Homarus* (Templeman, 1936, 1936a), *Callinectes* (Sandoz and Rogers, 1944) and *Palaemonetes* (Broad, 1957, 1957a). In spite of many reports of abnormal larvae and the provocative suggestions of Heegaard (1953) regarding penaeid development, documentation of variation in development of decapods in nature, in the form of either analyses of the frequency of occurrence of abnormal larvae or careful studies of the development of the abnormal larvae, is lacking. It is the belief of the author that variation in development which may be experimentally induced is also within the capability of the species in nature.

Although the differences between the structure of Lebour's larvae and those of Beaufort *T. floridanus* are not extreme, the difference in size of larvae of similar structure is at least suggestive of taxonomic distinctness. This, if taken in conjunction with the difference in color patterns of the larvae, results in reasonable doubt of the specific identity of Lebour's larvae.

SUMMARY

1. Larvae hatched from eggs carried by three female *Thor floridanus* Kingsley were reared in the laboratory at Beaufort, N. C. These larvae differed in size and color, structure and mode of development from *Thor* larvae, presumably of the same species, from Bermuda plankton.

2. If fed a diet of either *Nitzschia closterium* or *Nannochloris* sp., the larvae metamorphosed at the time of the 8th ecdysis, about 16 days after hatching. The first molt occurred from two to three days after hatching. The frequency of molting was once every 1.9 to 2.0 days.

3. The larvae of *T. floridanus* are described.

4. Because the number of individuals and the limited time during which larvae were available did not permit a greater variety of culture conditions, it cannot be said that the variation in mode of development between present and Bermuda larvae is not within the range of capability of a single species. On the other hand, differences in color and size of larvae of similar structure are suggestive of differences of taxonomic importance.

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PRELIMINARY STUDY OF BEHAVIOR OF NEWLY HATCHED OYSTER DRILLS, *UROSALPINX CINEREA* (SAY)[1]

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INTRODUCTION

Dense populations of *Urosalpinx cinerea* inhabit coastal waters on the east and west coasts of North America and in Great Britain. Bottoms in these areas teem with young of these muricid prosobranch gastropods during posthatching periods (Fig. 1). A large percentage survive to adulthood, favored by paucity of enemies and a high degree of adaptation to their native coastal marine communities. Casual field observations suggest that though lacking a free-swimming stage, newly hatched young may be dispersed some distance from parental areas. Soon after emergence from the egg case they locate and perforate young living bivalves in the vicinity, inflicting serious damage which is particularly evident on commercial oyster grounds.

Some studies have been reported on orientation reactions and other behavioral patterns of adult *U. cinerea* (Carriker, 1955b), but no investigations of this kind

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have been published on newly hatched drills. This paper contributes preliminary information on behavior in the laboratory of newly hatched *U. cinerea*, continuously isolated from older drills, to such physical environmental factors as water current, light, gravity, various surfaces, and evaporation; and to such biotic factors as attractants from newly set bivalves (Venus or hard clams, *Mercenaria mercenaria*). In addition some predator-prey interactions between these two mollusks are described, behavior in the laboratory is related to that in the field, and comparison of orientation reactions in young and adults is discussed. Newly hatched drills isolated from parents should permit observation of a behavior which is largely instinctive (Thorpe, 1957).

METHODS

Adult *Urosalpinx cinerea* were gathered by hand at low water in Bogue Sound in the vicinity of the Institute of Fisheries Research. They were numerous on rock jetties, shell accumulations, and wooden piling heavily encrusted with oysters *Crassostrea virginica* (Chestnut, 1956) and were found in salinities approximating those of sea water entering the laboratory. Over the year, salinities in Bogue Sound fluctuate between 20 and 36‰ (Chestnut, 1952; Chestnut and Fahy, 1952). All newly hatched drills (Fig. 2) used in this study were hatched in the laboratory from egg cases oviposited in running sea water tanks in the laboratory and from egg cases collected in the field with the adult drills. Hatching commenced 19 to 22 days after oviposition at temperatures ranging from 22 to 31°C and in salinities fluctuating between 31 and 36‰. Newly hatched drills averaged about one millimeter in height (also see Hancock, 1956). Adult drills were fed principally on young oysters (*Crassostrea virginica*) collected in Bogue Sound. Because of the need in behavior studies for normal active animals, newly hatched drills were maintained in the laboratory under as nearly ideal conditions as possible in running filtered sea water (in a flow of approximately 0.5 liter per minute) in several two-liter glass containers arranged horizontally in step arrangement (Fig. 4). Sea water flowed from a small settling tank into the bottom of the filter tank (modified from one in use by W. E. Kelley, pers. com.) passed slowly upward through a sedimentation chamber, and then through a two-inch layer of smooth sand and shell of particle size ranging from 1 to 3 mm. in diameter supported on a perforated wooden base overlaid by plastic screen of approximately one millimeter pore size. From the filter tank sea water flowed into the drill containers by way of a system of siphons (7.5 mm. inner diameter) set in series and so placed that water drained from the middle of the container. In this way drills could not reach the siphon except by crawling under the meniscus and floating in the water. A check siphon draining the last container controlled the level of water in all the containers. The inverse filter proved effective and easy to clean: corks in the bottom were pulled and fresh water was flushed through the sand without dismantling the filter. Running sea water was pumped (hard rubber pump) from Bogue Sound to, and distributed in, the laboratory directly in plastic pipe. The plastic (polyethylene) was non-toxic even to veligers of Venus in still-water cultures. This system of culture, though seemingly unnecessarily involved

was dictated by the capacity of young drills not only to crawl actively away from the bottom and toward the light over all surfaces including the undersurface of the meniscus, but to cling tenaciously to and float on minute bits of debris. Furthermore they expand the foot fully (about 7/8 the height of the shell, and proportionately larger than in adults), using it as a flotation device which permits them to remain suspended in water clinging to small sheets of mucus and aggregates of detritus. Newly hatched drills were provided bivalve shells heavily encrusted with minute sessile organisms (including oyster spat) for food. Clean shells were suspended in the water from the Institute pier periodically through the summer to provide these. Even under these conditions many drills floated through the culture system, transported by water flow on suspended objects. To minimize this loss effluent from the last drill container was run through a series of low enamel trays filled with shell heavily coated with oyster spat. Drills tended to accumulate on food organisms and could be brushed back into drill containers from time to time. Siphons leading from drill containers were not covered with screen since mesh sufficiently small to retain newly hatched drills soon fouled with organic films. Some young drills exhibited a strong tendency to crawl out of water on the sides of containers even under the most ideal laboratory conditions. These were brushed back two or three times daily.

Several thousand young drills were hatched and maintained in the laboratory for these experiments. Growth of young laboratory drills compared favorably to that of populations living in the field on native oysters and associated organisms. In one series of observations during the course of which some 900 newly hatched drills were examined closely, we found two *U. cinerea* whose shells were completely white and devoid of pigment, the eyes were jet black, and the soft parts were a creamy white color with flecks of darker cream. Later five similar "albino" drills of the following heights were discovered in the field, 6.5, 7.4, 8.3, 11.9, and 15.0 mm. During these behavior studies we found in the laboratory about a dozen young drills 1.1 to 1.4 mm. high which had been bored by other young drills and killed, indicating that even under our favorable culture conditions some cannibalism occurred.

Recently set Venus were cultured in the laboratory using procedures modified after those employed by Loosanoff (1954) and his associates. This represents the first time that Venus larvae have been cultured successfully to post-setting stages in the laboratory in areas other than the northeastern United States. Pure cultures of phytoplankton used in starting food cultures for Venus larvae were kindly contributed by W. A. Chipman and T. R. Rice, U. S. Fish and Wildlife

FIG. 1. A dense population of various stages of *Urosalpinx cinerea* on a cluster of oysters. Embryos in egg cases may be seen in the lower right hand corner. Two newly hatched drills (about 1 mm. in height) are shown in the center on an adult drill. ×5. FIG. 2. Newly hatched *Urosalpinx cinerea* (about 1 mm. in height) crawling over *U. cinerea* egg cases containing early embryos. The spire of an adult oyster drill is seen at lower left. ×10. FIG. 3. Characteristically smooth bevelled perforation by young *Urosalpinx cinerea* in young *Crassostrea virginica* is illustrated in the center of the photograph. *U. cinerea* egg cases are located in the lower left and an adult oyster drill is shown in the background behind the young drill in the center of the photograph. ×10.

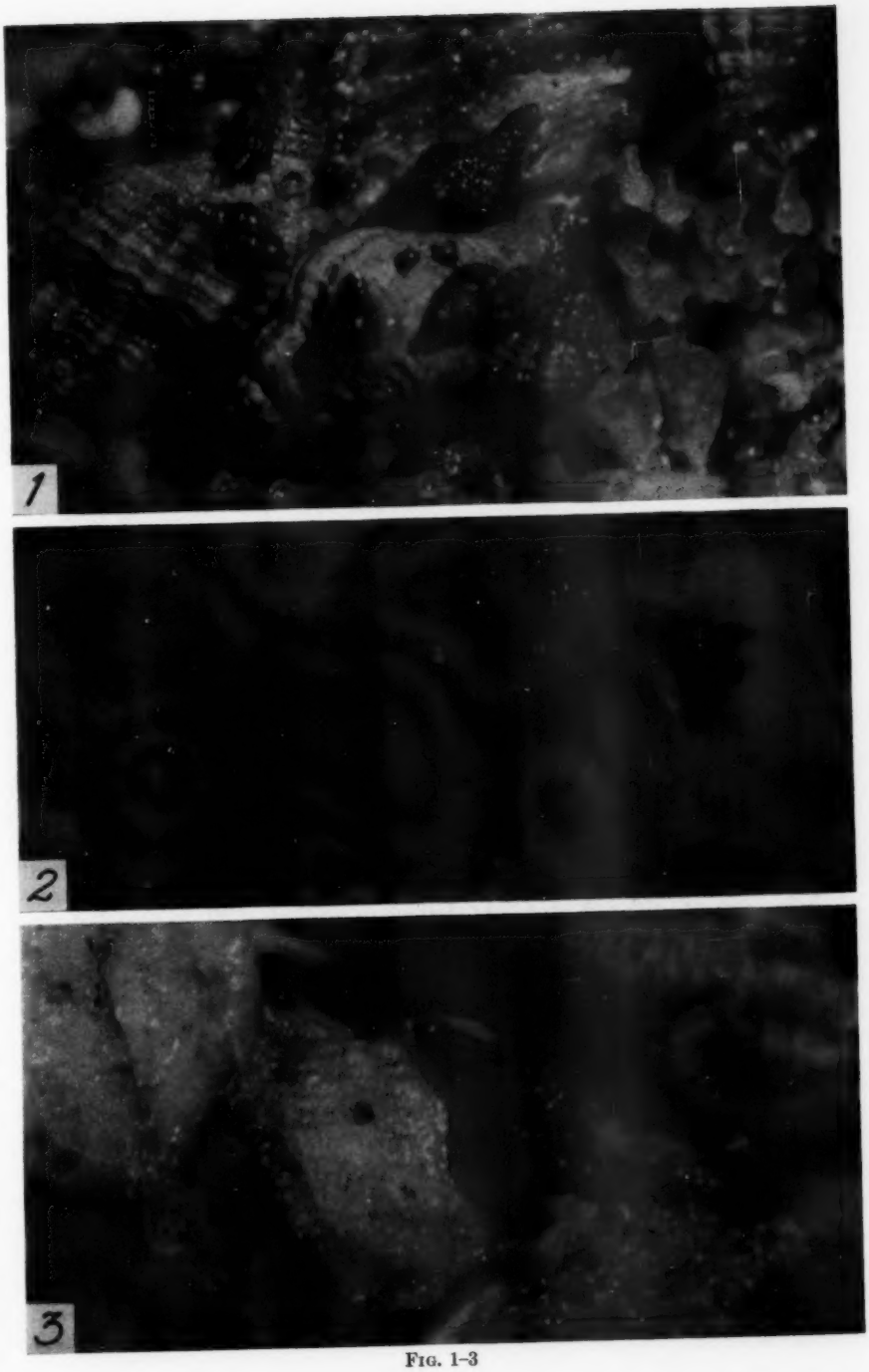


FIG. 1–3

Fishery Radiobiological Laboratory, Beaufort, North Carolina. Large populations of Venus grow natively in Bogue Sound (also see Chestnut, 1952).

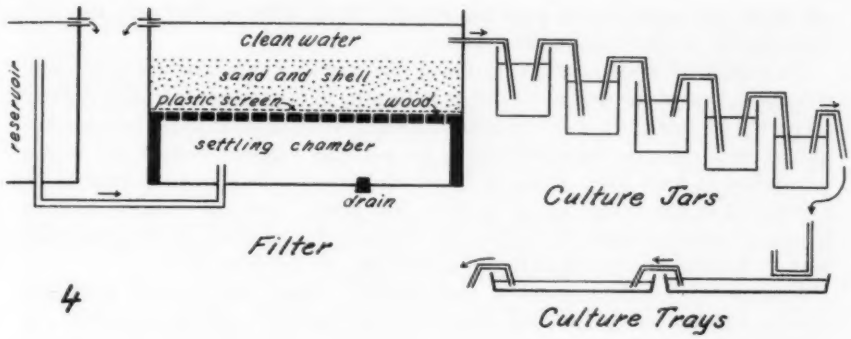
Specific methods employed in the study of various orientation reactions and other behavior will be described under appropriate headings in the following sections.

OBSERVATIONS

Orientation Reactions to Physical Factors

WATER CURRENTS.—Influence of water flow on movements of newly hatched drills was studied in a smooth wooden trough 8 feet long, 3.5 inches wide, and 3 inches high closed at the ends except for inflow and outflow perforations. A baffle was interposed below inflow tubes to equalize flow down the trough. The trough was coated with hot paraffin. Current velocity of sea water was determined by timing the rate of movement of a carmine suspension down the trough with a stop watch. During the experiments salinity of the sea water was approximately 35‰, temperature fluctuated between 27° and 30°C, and current velocity of sea water down the middle of the trough varied from 1.8 to 2.2 cm./sec. In a two-day check no young drills were transported into the laboratory in the running sea water system which delivers unfiltered sea water directly to laboratory water tables without interposition of a reservoir. In the following four experiments the reaction of drills to current was studied in a variety of combinations of light, darkness, and presence and absence of food.

Experiment A.—Three large living oysters bearing about 200 newly hatched drills (1.3 to 1.7 mm. high), numerous drill egg cases, and a heavy growth of small sessile organisms were placed some 17 cm. downstream from the inflowing water in the trough. Five large Venus shells covered with oyster spat ranging in diameter from 1 to 3 mm. were stationed one meter, and another similar set, two meters downstream from the drills and adjacent to the overflow. Normal laboratory daylight reached the trough: At the start of the experiment at 1600 hours, measurements with a Weston light meter gave readings of 800 foot candles at the overflow and 200 foot candles at the inflow. Thus an increasing light gradient was present downstream during the early and late stages of the experiment, which ran for 16.5 hours. At the end of seven hours 20 drills were found upstream and 34 downstream from the oysters; and after 16.5 hours, four drills were upstream and 108 downstream, 17 of the latter occurring on the screen. This distribution may be explained in the following way: A few drills crawled downstream. A conspicuous majority, however, crawled upstream, simultaneously creeping obliquely up the sides of the trough to the water-air interface. There a few crawled out of water, while the remainder crept outward under the meniscus. The latter were carried downstream, some sinking along the way, others passing over the overflow into the screen. On the bottom of the trough drills were attracted to and tended to concentrate on oyster spat; at the termination of the experiment, of the 108 drills collected downstream from the oysters, 53 were found on spat. Some drills were transported down the trough on bits of detritus. In one case a fragment of decaying vegetation entered the trough in the running



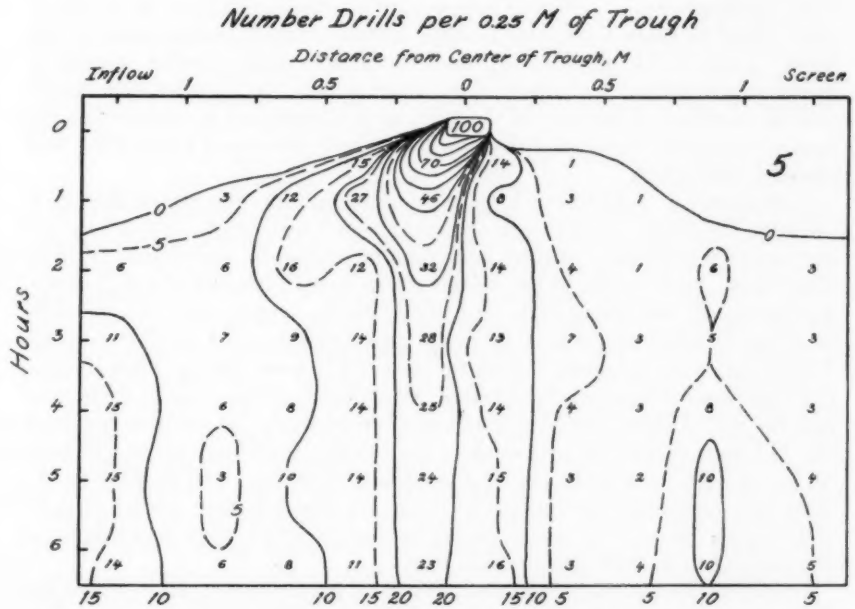


FIG. 4. Diagram of sectional view of the filter, culture jars, and culture trays used in the laboratory culture of young *Urosalpinx cinerea*. FIG. 5. Distribution of 100 newly hatched *Urosalpinx cinerea* up- and downstream from the center of a trough in response to current velocities ranging from 1.8 to 2.2 cm./sec., in the absence of light and of food, at approximately hourly intervals. Lines connect equal densities of oyster drills: solid lines, multiples of ten; broken lines, intermediate densities. Water flow moved from left to right. A screen under the overflow caught drills floating under the meniscus.

sea water and came to rest over the oysters. Three drills crawled onto this and were floated downstream to the overflow.

An incidental observation made during the course of this experiment is worth recording. One of the oysters (3 inches in length) in the trough extended slightly above the surface of the water. During the night while ejecting water between the valves, as is done so characteristically by partially exposed oysters, the oyster catapulted at least three young drills which were crawling near the edge of its valves out of the trough as far as three feet away. This may readily occur in intertidal zones as tides rise or fall over oysters. Young drills so projected may land on floating debris and be ferried away from the hatching area.

Experiment B.—Experiment A was repeated except that heavily encrusted oysters and 200 newly hatched drills were placed just inside the overflow and oyster spat were placed at one- and at two-meter intervals upstream from the drills. Observations were started at 1115 one day and concluded at 0915 the next. At the end of 6.25 hours one drill had crawled two meters and eight drills, one meter upstream; and 32 drills had been washed into the screen under the overflow. After about 15 hours there were 17 drills upstream and 171 downstream in the screen. Drills which moved away from the oysters again crawled principally upstream and toward the surface of the water so that the majority were floated over the overflow. A minority of the drills, as in Experiment A, remained on the oysters, presumably attracted there by food. Snails were most active when first placed in the trough.

Experiment C.—Experiment A was repeated except that oysters and drills were placed in the middle of the trough and oyster spat were located one meter up and one meter downstream, all light was excluded during the experiment (1015 to 2245 hours), and current in the trough was reversed after the first 6.25 hours. Approximately 300 young drills were placed on the oysters. After the first 6.25 hours seven drills were found on oyster spat upstream and 10 drills on that downstream from the oysters. After the second 6.25 hours during which the current was reversed simulating reversal of tidal flow, total drill count on these same spat for the 12.5-hour period was 14 and 17 respectively. At the termination of the 12.5-hour period (approximately a tidal cycle), there were about 100 drills on the oysters, 99 drills to one side (originally upstream), and 88 drills to the other side (originally downstream). During the first 6.25-hour period 21 drills collected on the overflow screen; during the second period when the current was reversed, 11 more drills accumulated there.

In spite of the absence of light drills continued to crawl under the meniscus and to float downstream. As in Experiment B locomotory activity was more pronounced during the first half of the experiment. Although most drills tended to crawl upstream, their floating behavior counteracted this and the majority actually moved downstream. In estuaries where tidal currents are constantly reversing, but in which net non-tidal water movements are often directed upstream along the bottom, one might expect drills to crawl down the estuary against net non-tidal flow. Actual drill distribution in these estuaries shows that drills follow the salinity barrier during dry periods (Carriker, 1955b), moving

up-bay as far as lethal salinities permit. This suggests that, as in these experiments, young drills crawl onto floating objects and are transported gradually toward the head of the estuary.

Experiment D.—This experiment was designed to demonstrate the movement of drills in moving water in the absence of food and in the dark over a period of 6.25 hours (1010 to 1625 hours). The trough was thoroughly cleaned and 100 active young drills, 1.2 to 2.0 mm in height, were placed in the middle. Every half hour the trough was uncovered for a brief period and a count of drills was made in one-quarter meter segments upstream and downstream from the center of the trough. Under these conditions the bulk of the drills showed a definite upstream movement, though considerable variation was evident in the rate of movement of different individuals in the population (Fig. 5). The slight aggregation of drills found 0.75 to 1 m. downstream resulted from floating drills which sank to the bottom before reaching the overflow. At the end of the experiment 62 drills were distributed upstream and 38 downstream from the center; five were carried into the screen. Of the drills crawling upstream, 14 moved over one meter, and three crawled a distance of 1.25 meters. It was possible to determine the rate of crawling of the most active drills over distances ranging from 0.5 to 1.25 meters shortly after introduction into the trough; three drills moved at the rate of 0.77 cm./min. and four at 0.83 cm./min.

Many newly hatched drills thus exhibit a strong tendency to crawl directly upstream against the current. According to the classification of Fraenkel and Gunn (1940) this orientation reaction, a directed one in which the animal moves directly toward the source of the stimulation, may be characterized as positive rheotaxis. That at these current velocities this response is not exhibited with equal intensity by all newly hatched drills is demonstrated in Figure 5. A few drills even moved with the current. It is possible that faced with stronger current velocities a greater proportion of newly hatched drills would orientate. Federighi (1929, 1931) found that a current of 1.25 to 7.6 cm./sec. stimulated adult oyster drills to turn immediately into and move against the current; and that speed of the current did not affect the rate of creeping. Removal of eyes and tentacles did not interfere with the rheotactic response. Stauber (1943) determined that at current velocities as low as 0.2 cm./sec. adult *Urosalpinx* no longer exhibited a positive response to current and even moved downstream. In our observations the tendency of newly hatched drill populations to move into the current was masked by their rather strong tendency to creep upward. It is likely that in the field wherever surfaces extend from the bottom young drills will behave the same and move upward as well as into the current.

LIGHT.—In the course of rearing newly hatched oyster drills in the laboratory in light intensities under 800 foot candles, we observed marked aggregation of young drills on the sides of culture containers facing the light. In order to test this orientation reaction quantitatively we made use of the following apparatus: an aquarium 18 inches long, 9 inches high, and 12 inches wide, with black slate bottom and ends, was covered with opaque materials except for a window 4 inches high along one side at the bottom.

Experiment A.—Natural daylight coming through laboratory windows was utilized. The aquarium was filled to a depth of four inches with clean cotton-filtered standing sea water of salinity 35‰. Filtered sea water was employed in order to eliminate small food organisms which might enter in the water and tend to eclipse the orientation of the drills to light. Some 900 drills, 1.0 to 1.6 mm. high, were placed on the center of the bottom of the aquarium with about 100 partially hatched drill egg cases. The experiment ran from 1030 to 1820 hours and during this time temperature of the sea water rose from 27° to 30°C, and the quantity of light fell from 50 foot candles at the start of the experiment to four foot candles at its termination, though the light intensity averaged about 40 foot candles for about the first six hours. No aeration was provided. At the end of eight hours the aquarium was uncovered and the distribution of drills was recorded. Thirty-eight per cent of the drills remained among the egg cases and 62 per cent moved away. Of those crawling away, 98.6 per cent moved definitely toward the light source.

Experiment B.—In this test the same apparatus was used but the light intensity was increased. A 150-watt electric light bulb in a standard light reflector was placed one foot away from the center of the window in the aquarium; 800 foot candles registered on the glass at the center, and 75 foot candles at each end of the window. Fresh cotton-filtered sea water was added to the aquarium, and egg cases and 800 of the drills utilized in Experiment A were introduced. Salinity and temperature remained the same. The experiment ran for 7.5 hours (1000 to 1730 hours). A survey of distribution at the end of the observations disclosed that 14 per cent of the drills remained among the egg cases and 86 per cent moved away; of the drills which crawled away, 98.5 per cent moved toward the light source. Thus a greater percentage of original drills moved away from the center in Experiment B than in Experiment A.

Experiment C.—Full outdoor illumination was used. The aquarium was filled with cotton-filtered sea water to a depth of four inches and placed on a lawn outside the laboratory. About 150 newly hatched drills were deposited on the bottom in the center of the aquarium about two inches from one end. All of the aquarium but five inches of the top at the opposite end was covered with opaque black cloth. Aluminum foil was wrapped over the cloth to reflect light thus keeping the aquarium cool. The experiment ran for two hours (1135 to 1335 hours) during which time temperature of the water climbed from 28° to 30.8°C. Salinity was approximately 35.3‰. The day was bright and cloudless with relatively no breeze. Light was very intense and could not be measured with the light meter available. The aquarium was shifted periodically to keep the exposed end fully lighted as the sun swung across the zenith. At the end of the two-hour period the drills were distributed as follows: of the 56 per cent which moved toward the lighted zone, 9 per cent moved into full light, 35 per cent remained in the shadows at the edges of the lighted zone, and 12 per cent moved about halfway to the lighted zone. Twenty per cent moved away from the lighted zone, and 24 per cent remained in the original position.

These three experiments indicate that newly hatched drills are attracted to

and move toward rather strong light, but tend to be repelled by intense outdoor illumination. In the classification of Fraenkel and Gunn (1940), adapted by Prosser *et al.* (1950), they are positively phototactic in moderate illumination and negatively phototactic in intense light. Drills possess a small eye on the outer margin of each of the pair of tentacles (Carriker, 1943) and it is presumably these which function as photoreceptors in the discrimination of the direction and intensity of light. Sizer (1936) and Cole (1942) in the laboratory and Stauber (1943) in the field observed that adult *Urosalpinx* tended to move away from intense and toward weaker light intensities. Sizer (1936) found that it was not until intensity of light was reduced to that of a 75-watt bulb that adult drills moved slightly toward light. In our experiments newly hatched drills were photopositive to much more intense light. As in the experiments on orientation of newly hatched drills to current, in these experiments on light many young drills climbed off the bottom, some crept out of water, and others crawled out under the meniscus.

GRAVITY.—A major difficulty in rearing young *Urosalpinx cinerea* in the laboratory results from the persistent efforts of many to crawl up the sides of the container out of water. In the following experiment, made after some preliminary trials, this orientation reaction was studied in relation to gravity.

Using the aquarium employed in the previous experiments, we placed two large elongated cotton-cheesecloth filters on the bottom along each side of the aquarium and brought in sea water (35‰) from a small settling tank in the laboratory at the rate of about 600 cc./min. Two glass-tube vertical overflows one at each end extending seven inches above the bottom of the aquarium carried off overflow water. Fifty newly hatched drills were dropped in the middle of each of two thoroughly cleaned and autoclaved glass 10 x 12-inch plates placed horizontally in sea water in shallow enamel trays and allowed to attach. Water was then siphoned out of the trays until drills were exposed. The glass plates were then carefully submerged in the aquarium in a vertical position, one on each side of the overflow tubes, the drills positioned on outer sides of the glass plates away from the overflows. Plates were elevated slightly above the bottom on glass rods to prevent drills which fell from crawling back up the glass plates. Strips of gauze were floated on the surface of the water adjacent to the vertical plates to block movement of drills crawling under the meniscus. The experiment ran for two hours (1515 to 1715 hours) and during this time temperature of the water remained at 30°C. The apparatus was completely blanketed in black cloth and the room was darkened to further exclude light. Slow flow of filtered sea water through the aquarium provided uniform distribution of oxygen and carbon dioxide and prevented stratification of any other substances which might alter the orientation reaction of drills to gravity; water flowed in such a pattern as to affect drills rheotactically only slightly, if at all.

Ten per cent of the drills placed on the vertical glass plates remained stationary; of 90 per cent which did move, 19 per cent were found on the bottom to which they fell either from the glass plates or from the meniscus. Or—which is least likely—to which they crawled. Eighty-one per cent crawled upward. In view of drills which fell from the plates this percentage probably should be

higher. The experiments demonstrate a strongly negative geotactic reaction on the part of these gastropods, and explain the persistent upward movements of drills in the previous experiments and observations. Federighi (1931) in Virginia discovered that adult drills exhibit a pronounced negative geotaxis, creeping upward when the temperature of the water rises above 10°C. This reaction was especially evident during the breeding season. The response persisted in a dark room with the eyes removed, and was not dependent on oxygen. On a vertical glass plate turned as a wheel, drills always turned so the siphon pointed up. A reversal of the response to gravity in adult drills at low temperatures, when they return to the bottom and assume positions just below the surface of sediments, has been reported (Carriker, 1955a; Galtsoff *et al.*, 1937; Stauber, 1943).

SURFACES. *Contact Reaction and Crawling.*—In still water newly hatched drills crawl over the bottom in a slightly zigzag course, moving from one side to the other in a searching pattern with the siphon tip extended a short distance beyond the tip of the shell. In a drill 1.8 mm. in height, for example, the fleshy siphon reaches about 0.1 mm. beyond the shell. They move readily over smooth surfaces such as glass or clean shell; when confronted with thin layers of fine detritus or sand they may creep over them or through them. In detritus, progress is very slow and they usually crawl submerged (two to three times the dorso-ventral dimension of the shell) as if to gain a better foothold in lower more compact layers. Progress over clean sand is rapid and direct. They can also plow over a smooth hard surface under fine sand to a depth at least twice their height. Thus recently set clams characteristically burrowed in superficial layers of sediment are exceedingly vulnerable to attack by these diminutive predators. Crawling drills secrete copious quantities of tenacious adhesive mucus which they leave behind in a ribbon-like trail. A strand of this attached to the foot will suspend the drill in water, though not out of it.

Young drills exhibit a very strong contact reaction. If upturned in detritus they experience considerable difficulty in righting themselves, and actively draw detritus under the foot until a small mass is aggregated which provides them a better hold by means of which to turn over. On firm surfaces when placed directly on their backs they right themselves in as short a time as two or three seconds. The foot is extended swiftly in the direction of gravity, the front portion the propodium, makes swift contact, and the shell is righted in a quick turn. Righting may be prolonged as long as a half minute if the drill has been upturned several times and experiences several unsuccessful attempts.

A young drill can adhere to the tip of a needle in less than a second and so tenaciously that it can be pulled through the water on it. A crawling drill affixes so firmly to solid surfaces that a forceful stream of water from a small rubber-bulb pipette is necessary to dislodge it. In an effort to determine how quickly and effectively young drills attach to firm substrata, we selected ten newly hatched drills at random and drew them one at a time into the stem of a small pipette with a rubber bulb, leaving them in the pipette for varying periods to determine minimum adhesion time. Drills landing on the foot attached in a minimum of one-half second; on the back, in three seconds. Less active drills consumed more

time. They started crawling as soon as the foot touched the glass, and could be prevented from adhering by water moving slowly back and forth in the pipette, though even under these turbulent conditions some attached.

Young drills tend to move about actively after a disturbance of any kind. In general they are much more active and respond more rapidly to environmental factors than adults.

Crawling out of Water.—When a newly hatched drill crawls upward toward the meniscus in a laboratory container it may move horizontally under the meniscus or may crawl out of the water entirely. As it creeps out it carries a large drop of water with it which collects principally around the foot and shell aperture in contact with the substratum. Probably because of its strong negatively geotactic response the snail seldom turns back into the water but continues moving upward. As it does so the film of water surrounding it evaporates. As this occurs the snail gradually retracts within its valve, and salinity of the water slowly increases becoming highly hypertonic to the fluids in the drill's body. The snail makes no effort to crawl again. It shrinks further into the shell until dehydration harmful to protoplasmic activity ensues and the mollusk dies. The opening of the siphon in the shell leaves a space through which evaporation continues, and a film of drying mucus secreted by the snail binds the shell to the substratum. In light experiment B in which 800 newly hatched drills were employed, 150 crawled out of the water on the sides of the aquarium in 7.5 hours. Many of these dehydrated and died even though returned to water.

An effort was made to determine the influence of living food and of water current on the proportion of drills which crawl off the bottom in the laboratory. Four jars of four-liter capacity each were employed. Sand-filtered sea water (about 35‰ salinity) was added to each, and food in the form of young oyster spat was placed in two of the jars. By means of appropriate siphons, running sand-filtered sea water (at the rate of about one liter per minute) was maintained in a jar with food and in one without. The other two jars, one with food and one without, were set aside with standing water. Approximately 100 newly hatched drills were placed on the bottom of each jar. The experiment was continued for about 18 hours, during which time air temperature ranged between 26° and 28°C. Distribution of drills in each jar at the termination of the observations is presented in Table I. These data show that drills were most active in running

TABLE I

Distribution of newly hatched oyster drills in jars under various conditions in the laboratory after 18 hours

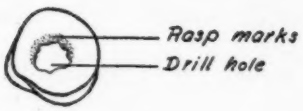
Conditions	Percentage distribution of drills in each jar			
	On food	On bottom off food	On sides under water	On sides out of water
Still water, no food	—	64	23	13
Still water, with food	65	17	13	5
Running water, no food	—	37	36	27
Running water, with food	90	5	0	5

water. In the absence of food a greater percentage crawled off the bottom in running water than off the bottom in still water. In the presence of food more drills crawled off the bottom in still water. Currents apparently not only stimulate increased locomotory movement on the part of young drills but also aid in dissemination of attractants from living food which direct drills to it. The smallest percentage of drills crawled out of water in the presence of both food and running water. Gradual continuous rise and fall of water level produced by means of appropriate siphons further reduces locomotion out of water. This emergent behavior may result from inadequate conditions imposed by artificial laboratory situations in which at least negative geotaxis and positive phototaxis in large part eclipse other orientation reactions, and thus in natural coastal areas may occur to a lesser extent. I have noticed for a number of years that adult oyster drills also tend to crawl out of water in aquaria, but as with the newly hatched individuals this emergent tendency can be almost completely blocked by introduction of running sea water (even in closed systems), living food organisms, and oscillation of water level. Moore (1938) likewise found that he was able to prevent the young of the marine gastropod *Purpura lapillus* from crawling out of laboratory containers by simulating tides, though the duration of the tide was of little consequence so long as the animals were uncovered at intervals.

The proportion of young drills which may be killed by exposure after crawling out of water in the laboratory or during low water in the intertidal zone is dependent upon such factors as nearness of the drill to water and the capacity of the substratum and sessile plants and animals thereon to retain or draw water by capillary action. Preliminary experiments demonstrated that young drills exposed on clean surfaces like glass or shell are killed in three hours or less in laboratory shade at temperatures in the vicinity of 27°C.

In a second experiment approximately 75 active young drills (1.0 to 2.4 mm. high) were allowed to adhere to each of three different vertical surfaces, the lower periphery of which dipped into running sea water permitting water to be drawn upward by capillary activity: a small square of clean glass, a Venus shell lightly encrusted with oyster spat and low coelenterates and bryozoans, and another Venus shell heavily fouled with living sessile organisms about one-quarter inch high. Drills were exposed on these surfaces in the laboratory for 6.2 hours (1600

FIG. 6–7. Newly set *Mercenaria mercenaria*, 240 μ in length, drilled by *Urosalpinx cinerea* 1.2 mm. in height. The perforation is approximately 100 μ in diameter. In most cases holes made by newly hatched drills are quite irregular, and in some instances the rasp marks of the radula are evident (Fig. 6). FIG. 8. A young *Urosalpinx cinerea* about 1.6 mm. high crawling under the meniscus of sea water. ×20. FIG. 9. A young *Urosalpinx cinerea* 1.5 mm. high drilling a young *Mercenaria mercenaria* 0.6 mm. in length. The Venus is held in the center of the foot by the oyster drill. The protoconch (valve developed in the egg case) and conch (shell added after hatching) portions of the valve are indicated. ×20. FIG. 10. Diagram of sectional view (upper portion) and vertical view (lower portion) of the apparatus used to study the response of newly hatched *Urosalpinx cinerea* to the external metabolites of very young *Mercenaria mercenaria*. Distribution of 92 oyster drills at the end of one hour in one experiment is indicated by numbers enclosed within broken-line areas.



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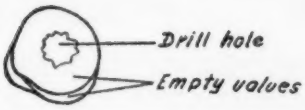

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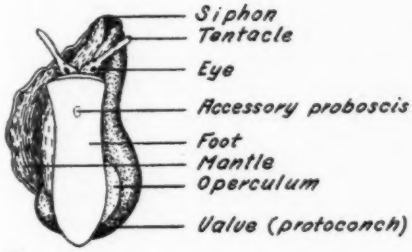

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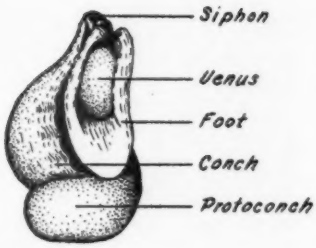

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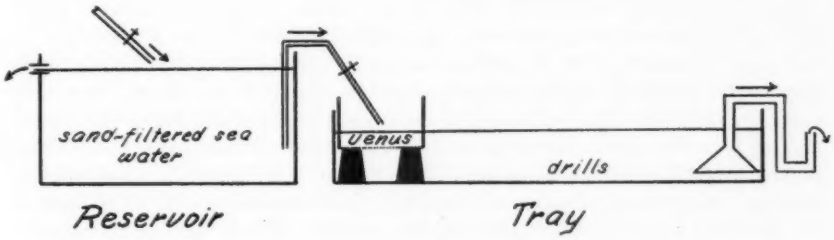

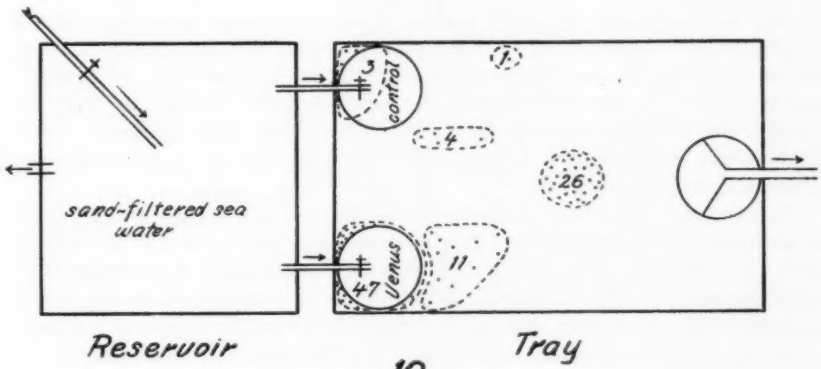

10

FIGS. 6–10

to 2215 hours), an average mid-intertidal exposure. Air temperature during the experiment ranged from 25° to 28°C, a light breeze was blowing, and high humidity such as would be found in the intertidal zone during a heavily overcast day prevailed. Rate of survival was determined by returning drills to running sea water for several hours. No drills survived exposure on clean glass or lightly fouled shell. Seventy-one per cent survived on the heavily fouled surface, indicating that sufficient water was held there to prevent dehydration.

In a final experiment approximately 50 newly hatched drills were allowed to adhere to each of six Venus shells lightly fouled with organisms. The shells were then placed on the sill of a north-facing laboratory window, simulating intertidal surfaces not in contact with water. Air temperature varied from 27° to 29°C., weather was clear and warm and a slight breeze was blowing. Survival after drying periods was checked by submerging drills in sea water for 8.5 hours. Duplicate shells and drills were exposed for one-, two-, and three-hour periods. Ninety-eight per cent of the drills on two shells survived one hour of desiccation; 89 per cent, two hours; and 61 per cent, three hours. This experiment indicates the degree of protection afforded young drills by sessile cover on intertidal surfaces. Negative phototaxis in bright illumination probably tends to direct them to heavily encrusted surfaces.

When crawling under the meniscus, as on other surfaces, newly hatched drills secrete copious quantities of mucus. This forms a sheet behind and sometimes about the foot which further buoys them. Considerable agitation of the water surface is necessary to dislodge and sink the snails. Complete retraction of the foot causes them to sink; however, they seldom retract the foot, crawling with this organ expanded (Fig. 8) until contact with a firm surface is effected. Drills may be moved about readily under the meniscus pulled on the mucus sheet with a needle. Dilute neutral red added to the surface of the water stains the mucus making it easily visible. Creeping under the meniscus seems to be a ciliary function entirely. Slow forward progress is achieved, but it is principally wind currents moving surface strata of water which propel these snails about. Drills as high as 7.7. mm have been seen crawling under the meniscus. When "finest carborundum powder" is dropped on the extended foot of an upturned drill, the grains move rapidly backward over the front portion of the foot where most rapid ciliary motion occurs. When forced to crawl under a submerged glass surface a snail moves forward quite smoothly, no muscular activity being evident at a magnification of 60 ×. In intertidal areas in nature it is probable that young drills can crawl under the meniscus only when water is relatively calm (Goldacre, 1949). Under these circumstances it is possible that they may be transported some distance from hatching areas.

TRANSPORTATION ON FLOATING OBJECTS.—In view of the suggestion made by some investigators (Federighi, 1931; J. R. Nelson, pers. com.; T. C. Nelson, pers. com.; Stauber, 1943) that young drills may be carried on floating debris by tidal currents, we next attempted to determine to what degree, if any, newly hatched drills crawl onto and are transported by various types of naturally occurring debris on coastal bottoms. There is no question that large quantities of

decaying *Zostera*, *Spartina*, many species of macroscopic algae, and other plants find their way into estuaries and embayments and are carried back and forth by tidal currents, dropping to the bottom during slack water. The critical question is whether young drills actively crawl onto objects which settle over them during slack water. Plant debris was collected in Bogue Sound and cut into two-inch lengths. A current sufficiently swift to float and move these fragments was established in the trough used in the current-velocity experiments. Fifty newly hatched drills were placed in the trough within a circle about three inches in diameter for each trial. Then 10 plant fragments were dropped gently over the drills and allowed to remain over them for ten minutes. Water current was gradually increased until the fragments floated away. The number of drills adhering to these was recorded. This procedure was carried out with debris of salt-marsh grass, *Spartina alterniflora*; eel grass, *Zostera marina*; and a much-branched macroscopic green alga, *Gracillaria* sp. In trials 1–6 (see Table II) drills were placed on the clean paraffined surface of the trough; in trails 7–10, in Venus shells encrusted with living organisms. In the latter the two-inch plant fragments were minced into pieces ⅛ inch long. During the experiments salinity of the water remained about 35‰, and water temperatures fluctuated between 29.5° and 31.0°C.

As demonstrated in Table II the proportion of drills crawling onto debris depends on a number of factors. Because of their active negatively geotactic reaction they climb onto objects that touch them and are higher than the substrate over which they crawl. Thus when drills were placed on the flat trough surface more crawled onto *Spartina* and *Zostera* fragments than onto the slender

TABLE II

Percentage of drills (50 drills per trial) crawling onto and transported by plant fragments of different kinds under various ecological conditions

Trial	Substratum	Plant fragments	Percentage drills transported
1	Clean paraffin	Clean fresh *Gracillaria* sp.	12
2	"	Clean fresh *Gracillaria* sp.	21
3	"	Clean yellow water-logged *Spartina alterniflora*	34
4	"	Clean yellow water-logged *Spartina alterniflora*	27
5	"	Old green blades of *Zostera marina* free of living organisms	22
6	"	As above but with some living sessile organisms	49
7	Venus shell with living sessile organisms	Clean yellow water-logged *Spartina alterniflora*	0
8	"	Old green *Zostera marina* with some living sessile organisms	0
9	"	Minced clean yellow water-logged *Spartina alterniflora*	18
10	"	Minced old green *Zostera marina*	6

much-branched *Gracillaria*; when in the cupped surface of a Venus shell, in which only slight contact was effected with the fragments, very few adhered. Drills creeping over the surfaces covered with living food organisms were not attracted to fragments devoid of these, and even those with some sessile animals did not compete favorably with food organisms attracting them on Venus shell. Conversely plant fragments encrusted with living sessile animals attracted more drills than similar clean fragments.

Under the conditions of these laboratory experiments as many as 49 per cent of a newly hatched drill population crawled onto plant fragments during "slack" water and were swept downstream by flowing water. Some years ago, T. C. Nelson (pers. com.) observed the transport of newly hatched *U. cinerea* a distance of at least 40 feet on *Zostera* floating in a tidal stream. If this behavior is characteristic of newly hatched oyster drills in nature—and there is no reason to suspect that it is not—it is clear that this animal, in spite of the lack of a planktonic veliger, is dispersed far more widely and in greater abundance in its early post-hatched stages than has been thought possible up to the present time. This type of passive emigration probably accounts for much more dispersion of the species than phoresis, and may provide an additional explanation for the "sudden appearance" of high concentrations of oyster drills on oyster grounds (Carriker, 1955b).

Orientation Reactions to Biotic Factors

Survival of newly hatched *U. cinerea* is vitally dependent upon successful identification, location, and penetration of food organisms. On oyster grounds they are known to prey upon newly set oyster spat, among other organisms, immediately upon emergence from the egg case. Numerous records are available on the rate of this destruction (Carriker, 1955b). The oyster being an epifaunal organism, is continuously vulnerable to attack. The predator-prey association of oyster drills and Venus, an infaunal bivalve living in large concentrations in coastal marine communities often associated with oyster drills, has received scant attention. Incidental unpublished observations indicate that among burrowing bivalves younger dissoconch stages are attacked when partially exposed in the sediments shortly after metamorphosing from veliger stages (Carriker, 1955b). In this section preliminary information will be presented on the orientation reaction of newly hatched oyster drills to recently set Venus, on the drilling and feeding behavior of newly hatched drills on newly set Venus, and on the rate of predation of newly set Venus by newly hatched oyster drills.

EXTERNAL METABOLITES OF NEWLY SET VENUS.—Newly hatched oyster drills maintained in either still or running sea water containers in the laboratory readily locate newly set oysters and Venus when these are added. Drills seem to orient to attractants (possibly in the category of external metabolites considered by Lucas, 1955) emitted by prey (also see van Dongen, 1956). Characterization of this external metabolite, the manner of its identification by the drill (chemoreception), and the manner of confirmation by the drill of the immediate presence of prey after it has been directed to it have yet to be reported. Preliminary

research by Blake (pers. com.) has demonstrated that an oyster's relative attractiveness to adult drills is directly proportional to its metabolic rate (as indicated by rate of utilization of dissolved oxygen). Generally the gradient of concentration of the chemical stimulus, however, does not seem sufficiently steep to direct drills straight to prey. Their orientation reaction for the most part is a positive chemoklinotactic one (Fraenkel and Gunn, 1940), in which they crawl toward the stimulus creeping from side to side, apparently comparing the intensity of the stimulus at the extremes of the locomotory oscillation. The few preliminary investigations available on chemotaxis in adult drills have already been reviewed (Carriker, 1955b); no research of this kind has been performed with newly hatched *U. cinerea*.

In an effort to study the orientation reaction of newly hatched drills to external metabolites of newly set Venus I employed a device (Fig. 10) patterned somewhat after the one used by Haskin (1950) in a similar study of the response of adult drills to oysters. Newly hatched drills were placed in the center of a pyrex glass tray (12 inches long, 8 inches wide, and 1.8 inches deep). Two stainless steel screens three inches in diameter, partly submerged in sea water 1.5 inches deep, and supported in tripod fashion on rubber stoppers were placed in the corners at one end of the tray. Clean sand-filtered sea water flowed slowly in equal volumes through each of the screens. Recently set Venus were added to one screen and the other was left blank as a control. Sea water was siphoned from the opposite end of the tray through a small inverted funnel and check siphon which removed water from near the bottom. Pattern of water flow was determined by adding finely suspended carmine at the inflows. Water passing through the screens moved slowly down the tray past the drills at the center to the funnel. Drills thus were given an opportunity to discriminate between clean sand-filtered sea water and similar water bearing external metabolites of Venus. Since velocity (even though very low) was similar in both currents, rheotaxis was disregarded. However, because of the strong positive phototactic response of these snails, it was necessary to exclude light from the apparatus. This was done by covering with cardboard and black cloth and darkening the windows in the laboratory. The covering was removed briefly at intervals to permit determination of drill distribution.

After a number of preliminary experiments had been run to determine optimum conditions for testing chemotaxis, the following experiment typical of the series was performed. Ninety-two drills (1.0 to 2.1 mm. high) were placed in the center of the tray and about 10,000 young Venus (225 μ to 1.4 mm. long), in one of the screens. Water flow through each screen was regulated to about 15 cc. per minute. Salinity during the experiment was about 35.5‰ and temperatures averaged 29°C. Five minutes after the drills had been positioned in the tray, 24 had moved to the vicinity of Venus, three to the blank, and none elsewhere. At the end of one hour 28 per cent of the drills remained in the center of the tray. Of the drills which moved away from the center 88 per cent moved directly toward, and the majority under, the screen supporting Venus, and 12 per cent crawled to the blank (Fig. 10). Under these conditions, then, young drills exhibited a pronounced

reaction to the external metabolites of small bivalves in the screen. That some drills moved toward the blank may be explained by the fact that water entering the apparatus was pumped to the laboratory through plastic pipe moderately fouled with young oysters and other organisms and some of their metabolites undoubtedly entered the apparatus. Also it is possible that drills located nearest the control flow when first placed in the tray may have been more actively stimulated by current flow than by Venus metabolites.

DRILLING AND FEEDING BEHAVIOR OF NEWLY HATCHED *UROSALPINX* ON NEWLY SET VENUS.— The following description is a composite of a number of observations of newly hatched drills attacking young Venus. Prey and predators were placed in a No. 100 stainless steel screen in sea water in a petri dish and examined under 10×, 30×, 60×, and occasionally 80× magnifications of a binocular microscope. Illumination was provided by a slender shaft of light from a microscope illuminator directed under the specimens by the substage mirror. The screen blocked excessive glare, yet permitted sufficient illumination to follow drilling and feeding carefully. Active young drills are voracious feeders and not easily dissuaded from attacking and drilling prey, thus it was possible to observe the behavior expressed more clearly than in adult drills (Carriker, 1943). Details of the actual drilling process are being investigated and will be published later.

Clearest observations were made on a drill 1.5 mm. in height attacking a Venus 0.6 mm. in length. The drill embraced the clam in the sole of its foot, which displayed a surprising capacity to manipulate the clam and support it in the "desired" position. This is usually the middle of one valve, and explains why we found so many drill holes near the center of the valve. After attaching securely to the clam, the drill permitted me to turn it over slightly to expose a view of the foot (Fig. 9). As in the adult, the young drill extends its proboscis to the prey through a protecting tube formed by the inward overlapping of the propodial folds of the foot. After the drill has captured prey, it is difficult to dislodge or distract it from its food. In some instances young Venus made numerous futile attempts to crawl away from their captors. As indicated by muscular activity of the cephalic region of the snail, drilling alternated with rest periods. A perforation was drilled in about 45 minutes, then the drill extended its proboscis through the hole and began feeding. As valves of small clams are quite translucent, activity of the proboscis within the clam was clearly observed. Flesh was removed in about 45 minutes. The adductor muscles of the clam were the last to be rasped. During the last five minutes of feeding the drill removed these and the valves slowly gaped. When the flesh was entirely consumed the drill withdrew its proboscis, pulling it slowly through the tight-fitting hole. The inner diameter of the perforation was 101 μ, and it bevelled broadly externally to an outer diameter of about 152 μ. Action of the radula was also clearly visible within the valves of the prey, particularly after most of the flesh was removed. The highly pliant proboscis was readily moved over the interior of the clam. The radula slid typically back and forth over the odontophore as it scraped tongue-fashion over flesh. Passage of food particles down the esophagus was also visible.

RATE OF PREDATION OF NEWLY SET VENUS.—Unpublished observations (Gibbs

in Rhode Island, Carriker in New Jersey) are known in which significant quantities of very small Venus drilled by young oyster drills were encountered in the field, but rate of predation under controlled conditions has not been observed. Accordingly we designed a number of experiments to study this in the laboratory, using laboratory-cultured Venus and laboratory-hatched oyster drills. Drills and Venus were housed in new sand-scoured finger bowls (350 cc. capacity), some in still water, others in running water. Fine sand was added to the bottom of some of the containers with running sea water to permit young clams to burrow. All finger bowls were kept covered with glass plates. Drills in running sea water were maintained in finger bowls placed around a shallow enamel tray of three-liter capacity. Clean sea water was siphoned into this from a small settling tank; from the enamel tray water flowed into the finger bowls through small glass siphons (1.25 mm. inner diameter), and out of these through slightly larger siphons (2 mm. inner diameter) with inflows held away from the sides of the bowl. This arrangement reduced the chances for drills to escape. Salinities in the experimental dishes fluctuated between 34.8 and 36.1‰ and water temperatures between 22° and 31°C. In the running sea-water dishes Venus obtained their food from the water; in dishes with standing water a mixed culture of plankton, principally *Nannochloris sp.*, cultured in the laboratory, was added. Since young drills are so strongly negatively geotactic and so positively phototactic we experienced considerable difficulty in restricting them to the bottom of containers. Those crawling out of the water were dropped back two or three times a day. In several experiments not reported here, young drills crawled out of the water and dehydrated. Design of experiments and data on rate of predation are given in Table III. Under these conditions rates varied from one to 19 young clams drilled per young drill per day. These rates are too low because in most experiments a proportion of Venus (almost as many as those drilled) was killed and consumed without drilling, and the counts in Table III record only clams with definite drill holes in them. Although never observed, it appears that clams killed without drilling were attacked through gaping shells while they were crawling or siphoning, or possibly by suffocation with mucus which drills liberated in copious quantities. This mucus coated drilled valves and many undrilled gaping empty valves. Drills (ranging in height from 0.9 to 2.0 mm.) tended to attack the larger of the Venus present: minimum length of clams in the various dishes was 220–250 μ, and clams drilled in these dishes ranged in length from 250–950 μ. Perforations were generally made in the center of the clam valve; occasionally, on the umbone or at the junction of the valves. Holes bored by the youngest drills were irregular in outline suggesting that they might have been made by rasping and chipping away the fragile clam shell (Fig. 6), on the shells of larger thicker Venus and oysters the perforations bored by older drills were characteristically smooth and deeply bevelled (Fig. 3).

Addition of thin layers of fine sand to some of the predator-prey containers, permitting clams to burrow, did not seem to afford much protection because drills crawled through the sand to them (Exps. 13 and 14, Table III). Highest predation rate occurred in Experiment 6. In Experiment 7 a small drill was inad-

TABLE III

Rate of predation of young *Mercenaria mercenaria* by newly hatched *Urosalpinx cinerea* in the laboratory

Exp. No.	Length of Venus			Height of drills, mm		Exposure time, days	Culture conditions	No. Venus drilled per drill per day
	No. Venus	mm	No. Drills	At start	At end			
1	300	0.22–0.29	4	1.0–1.2	—	2	Still water	4.5
2	300	0.22–0.29	2	1.0–1.2	—	1	Still water	8.0
3	300	0.22–0.29	1	1.0	—	2	Still water	10.5
4	300	0.22–0.29	1	1.2	—	2	Still water	13.5
5	300	0.22–0.29	1	1.0	—	1	Still water	14.0
6	300	0.22–0.29	1	1.2	—	1	Still water	19.0
7	25,000	0.25–1.20	1	(?)	2.0	6 (?)	In large Venus culture, aerated	14.0
8	200	0.25–1.30	1	0.9	1.6	7	Running water	4.5
9	200	0.25–1.30	1	1.1	1.7	7	Running water	3.0
10	200	0.25–1.30	1	1.4	2.3	7	Running water	7.3
11	200	0.25–1.30	1	1.3	1.4	7	Running water	1.0
12	200	0.25–1.30	1	1.3	2.2	7	Running water	5.1
13	200	0.25–1.30	1	1.4	2.8	7	Running water and sand	6.8
14	200	0.25–1.30	1	1.3	2.2	7	Running water and sand	6.0

vertently introduced into one of the large Venus cultures (approximately 25,000 Venus) and left there a maximum of six days (possibly only three, since it is questionable when drill was introduced—the count in Table III is based on six-day exposure). This culture of Venus contained clams ranging in length from 250 μ to 1.2 mm. The drill when discovered measured 2 mm. in height. During its stay in the culture it bored Venus ranging in length from 350 μ to 950 μ, and the perforations in these varied from 100 μ to 160 μ in diameter, indicating the rate of growth of the drilling mechanism during that time. Growth rate data collected in Experiments 8–14 showed that most drills almost doubled their height (one drill did so, Exp. 13) in one week. The drill doubling its height consumed 48 Venus in the week. Maximum predation rates occurred in still water.

Although these experiments on predation were carried out only with young Venus as prey, they indicate the potential destructiveness of these tiny drills to bivalves even after they have developed the byssus and burrowed into sediments. In the presence of additional food species on natural bottoms it is likely that epifaunal food species would function as buffers to infaunal species, and that predation would be reduced. To what degree this might take place is not known.

Discussion

Behavior in newly hatched *U. cinerea* is determined to a marked degree by response to the combined and occasionally antagonistic effects of ecological factors. As only newly hatched oyster drills completely isolated from older drills were utilized in this study it is probable that the behavior they exhibited is essen-

tially instinctive. These snails are nicely adapted even at these early stages in the life history to the complexities of their coastal marine communities.

In the absence of food the response of newly hatched drills to current keeps them moving into flow over the bottom. Strong negative response to gravity and strong positive response to moderate illumination impels them upward from the bottom away from the danger of suffocation by suspended sediments which are densest immediately over soft bottoms, and onto available elevated food-bearing surfaces. This orientation reaction also brings them onto floating vegetation coming to rest over them on the bottom during slack water. In intertidal zones strong light keeps them under shaded objects, as we observed in natural areas in Bogue Sound during the summer. Agility and adhesiveness of the foot insure quick and sure anchorage to firm substrata of all sorts, including floating materials. Capacity of young drills to cling to floating debris promotes emigration and wider dispersion. Their capacity to hang under the meniscus, particularly in intertidal areas when calm water prevails, permits expression of this reaction, and also promotes wide distribution. Quick and marked response of young drills to food organisms insures location of this food and in combination with other responses also encourages dissemination. Drills which may hitch a ride on floating debris during slack water at one point devoid of food organisms, more readily crawl off at the following slack water some distance away in the proximity of food animals.

These behavioral studies indicate that young drills move about much more actively and widely over native grounds than was once supposed, and help to explain how large numbers of young drills may appear on oyster bottoms which did not appear to harbor them previously.

SUMMARY

1. Orientation reactions of newly hatched oyster drills, *Urosalpinx cinerea* (Family Muricidae, Subclass Prosobranchia, Class Gastropoda), to water current, light, gravity, various surfaces, evaporation, and external metabolites of newly set Venus (hard clam, *Mercenaria mercenaria*) under laboratory conditions are described. Predator-prey interactions of these two mollusks are also discussed.

2. In water temperatures between 27° and 31°C and at salinities in the vicinity of 35‰, newly hatched drills exhibited a pronounced positive rheotaxis, a strong positive phototaxis in moderate illumination and a negative one in intense outdoor light, a strong negative geotaxis, a rapid righting reaction, and a marked capacity to adhere to surfaces.

3. Newly hatched drills tended to crawl upward off the bottom, some vertically out of the water to dehydrate, and others horizontally under the meniscus. Locomotory progress under the meniscus was relatively slow, horizontal movement being effected principally by water currents. Presence of food, of water flow, and of oscillation in water level reduced the number of drills crawling out of water. Dehydration was greatly reduced on intertidal surfaces overgrown with sessile organisms.

4. Newly hatched drills crawled actively onto floating debris which settled

over them during slack water. In various populations as many as 49 per cent crawled onto plant fragments and were swept downstream. Wide dispersal is thus assured.

5. Newly hatched oyster drills exhibited a pronounced positive chemotactic response to external metabolites of newly set Venus. Prey were readily located. Once a clam was grasped in the foot it was not readily relinquished by the drill. Young drills are voracious feeders. A drill 1.5 mm. in height perforated a Venus 0.6 mm. in length in 45 minutes and rasped out the flesh in another 45 minutes. Predation rates varied from one to 19 young Venus drilled and consumed per young drill per day. Since young drills can crawl through superficial layers of sediment, newly set bivalves were not protected from them by burrowing shallowly. In nature buffer-food species probably reduce this predation rate.

6. These studies suggest that newly hatched *U. cinerea* crawl about much more actively and are dispersed much more widely than was once supposed.

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THE INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE METABOLISM OF MELANISTIC STRAINS OF *DROSOPHILA MELANOGASTER*[1]

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INTRODUCTION.—In recent years an increased interest in the evolutionary role of physiological differences of organisms has intensified studies in comparative physiology. Investigations have taken various expressions: some investigators have compared tropical and arctic animal species (Scholander *et al.* 1950, 1953); some have studied specimens of a single species as collected from various extremes of the geographic range (Tashian, 1956); and still others have examined closely related species found inhabiting the same area.

The present problem is concerned with the adaptive responses of melanistic forms of *Drosophila melanogaster* found in wild populations. According to Gloger's rule, dark insects inhabit cool humid regions, while lighter ones inhabit warm arid regions. The rule is supported by studies on carabid beetles (Netolitzky, 1931), vespid wasps (Zimmerman, 1931), coccinellid beetles (Dobzhansky, 1933) and *Drosophila melanica* (Ward, 1952). Dobzhansky (1933) notes from his studies that the high humidity seemed more important than low temperature as a factor correlated with heavy pigmentation. Kalmus (1945), studying adaptive responses of ebony versus wild type *Drosophila melanogaster* in population cages, found the ebony homozygote to increase in numbers when the temperature and relative humidity were dropped from 25°C. and 70 per cent R.H. to 12–15°C. and 10 per cent R.H. Kalmus (1941) found that ebony homozygotes were better able to withstand desiccation than light-colored flies and suggested that dark color would be an adaptation to drought and low temperature.

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In 1955 Jacobs began a search for melanistic forms of *Drosophila melanogaster* in wild populations. On July 28 a large sample of flies was collected from a grocery garbage can at Beaufort, North Carolina. When the samples were grown in half-pint bottles on agar-cornmeal-yeast-syrup medium seeded with Fleishmann's yeast, dark and light flies began to emerge after several generations. The dark form emerging most commonly had a markedly dark trident and scutellum and slight darkening of the sclerites in general, but was less dark than those of the mutant "black". This form is herein designated as dark. When dark flies are crossed with the mutant ebony the F_1 grown at 25°C. are intermediate between dark and ebony, and the F_2 are dark, intermediate, and ebony in 1:2:1 ratio. The gene for dark is therefore an ebony allele. This dark strain and a light strain were isolated from the same sample bottle by sib pair matings. The light flies have a tan body color and no dark trident and little darkening of the scutellum. When the dark and light flies are crossed, the resulting heterozygotes, when grown at 15°C., are all darker than the light parent, but when grown at 25°C., most of the heterozygotes are similar to the light parent. From the F_2 of a light × dark cross, sib pair matings were made and one light and one dark true breeding strain were selected for this study.

The strains were propagated by sib matings for six generations and were then kept in mass culture. Twenty females and four males were placed in half-pint milk bottles with about 50 cc. of Baker's modification of Carpenter's medium seeded with Fleischmann's yeast. Each day ten fresh females and two males were added and all the parent flies were transferred to a new bottle. These were incubated at 25 ± 1°C. in an incubator with temperature controlled by an ether-mercury precision thermoregulator, and air circulated with a twelve-inch fan. The incubator contained a tray of water to maintain humidity. The pupae used in the study were picked from the walls of the bottles. Adults used were shaken from the bottles one to two hours after emergence.

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MATERIALS AND METHODS.—The Q_{O_2} of light, dark, and heterozygous flies was measured by the direct method of Warburg. Q_{O_2} is defined as the microliters of oxygen consumed per milligram (wet weight) of fly per minute. Adults to be used were lightly etherized and five males or females were placed in a gelatin capsule which had been pierced with a hot insect pin to make about eight holes. The capsules were weighed with a torsion balance before and after filling in order to determine the weight of the flies. After the flies had apparently recovered from the ether, each capsule was placed in the center well of a small (5 ml.) Warburg flask. 0.2 ml. of potassium hydroxide was put in the space surrounding the center well. The flasks were attached to manometers and placed in a constant temperature bath. After a ten-minute equilibration period, the manometers were read at twenty-minute intervals for a period of not less than an hour. The Q_{O_2} of adults was measured at 13°, 18°, 22°, 27°, 30°, 33°, 36°, and 39°C., at relative humidity levels of 10 per cent and 98 per cent. The Q_{O_2} of pupae was measured in the

TABLE I

Oxygen consumption of various color strains of adult Drosophila melanogaster *at different temperatures*

Temp. °C.	Dark Strain			Heterozygotes (d/l)			Light Strain		
	No.[1]	Mean Q_{O_2} (ul/hr/gm)	S.E.[2]	No.[1]	Mean Q_{O_2} (ul/hr/gm)	S.E.[2]	No.[1]	Mean Q_{O_2} (ul/hr/gm)	S.E.[2]
13	8	12.57	0.605	9	15.99	0.740	8	14.85	1.121
18	10	27.28	0.924	10	47.94	0.396	10	30.88	0.788
22	12	24.12	1.129	11	46.40	2.153	11	24.60	1.417
27	12	44.46	2.027	8	59.91	4.314	12	55.75	2.434
30	11	43.41	3.800	8	60.49	6.330	12	55.78	2.714
33	8	59.92	2.157	7	78.99	3.779	8	78.49	2.949
36	16	71.42	3.675	12	76.48	6.091	16	78.32	3.850
39	8	121.50	7.355	9	36.10	2.253	8	115.15	13.083

[1] Number of determinations

[2] Standard error

same manner at 13°, 25°, 30°, 35°C. and 10 per cent and 98 per cent relative humidities. Humidity was controlled by using different concentrations of potassium hydroxide as described by Buxton and Mellanby (1934). An oxygen-consumption curve for the flies from the beginning of the time of pupation to the time of their emergence as adults was also determined.

Dry weights of adults were obtained by weighing a group of freshly killed adults, placing them in an oven at 110°C. for two days and then re-weighing the desiccated flies.

RESULTS. *Adult Respiration.*—The rates of oxygen uptake determined at different thermal levels for one-day-old adults of the three types used in this study are shown in Table I. At 13°C. the heterozygous flies utilized more oxygen per unit weight and time than either of the other two strains. The rate for heterozygous is significantly higher (at the 1 per cent level) than for dark flies but it is not statistically different from the metabolic rate of the light strain. Determinations at 18°C. showed marked physiological differences among the various strains studied. Heterozygotes have a metabolic rate which is significantly higher than that of dark flies. Q_{10} values for this 5° increase in temperature are as follows: heterozygous flies, 8.98; dark flies, 4.71; and light flies, 4.32 (Table II). A further increase in temperature of 4°C. did not increase the metabolic rate of these forms but actually resulted in lower mean Q_{O_2} values. When increasing the thermal level by a 5°C. increment (from 22°–27°C.) the Q_{10} for the light flies is 5.14, the dark strain, 3.39, and the heterozygous flies, 1.67. At this new temperature level of 27°C., the heterozygous flies no longer have a metabolic rate which is significantly different from that of the light strain, but both consume oxygen at a significantly higher rate than the dark strain (significant at the 1 per cent level). The mean metabolic rates determined at 30° are approximately the same for the three types of flies as they were at 27°C. Another 3°C. increase in temperature resulted in higher metabolic rates of all three types of flies. The means for the heterozygotes and light forms are significantly higher (at the 1 per cent level)

TABLE II

Q_{10} values of the metabolic rate of various color strains of Drosophila melanogaster

Temperature (C.)	Dark	Heterozygotes	Light
		Adult	
13°–18°	4.71	8.98	4.32
22°–27°	3.39	1.67	5.14
30°–33°	2.92	2.43	3.12
33°–36°	1.80	—	—
36°–39°	5.87	—	3.61
13°–22°	2.06	3.27	1.75
22°–30°	2.08	1.39	2.78
30°–39°	3.14	—	2.24
		One-day pupae	
13°–25°	2.08	2.55	1.86
25°–30°	3.81	3.30	4.87
		Four-day pupae	
13°–25°	2.97	4.10	3.95
25°–30°	3.27	—	1.32
30°–35°	1.08	3.43	1.37

than for the dark strain. The Q_{10} of the heterozygotes is 2.43 and for the light flies and dark flies the figures are 3.12 and 2.92 respectively. At 36°C. the mean values for all three types of flies are very similar, but at 39°C. distinct differences are evident. Unlike the light and dark strains, a temperature of 39°C. for one hour is lethal to most of the heterozygotes and thus the Q_{O_2} value represented in Table I for this type of fly represents oxygen used for one hour regardless of the death of some of the flies in any one capsule. Death of the flies at a high temperature slightly below 39°C. is similar to results of Soltavalta (1947) who reported that in saturated atmospheres *Drosophila melanogaster* died at 39°–40°C. The dark strain had a slightly higher rate of mortality than the light strain but this difference was not significant.

Sex Differences.—Comparative data of the metabolic rates of males and females of each strain are found in Table III. No tendency was observed for one sex to consume oxygen consistently at a higher rate than the other throughout the entire temperature range. However, it appeared that at higher non-lethal temperatures the males of all three strains used more oxygen than females, whereas at intermediate temperatures the reverse situation generally was observed. With a further drop in temperature, each strain responded differently. For example, at 13°C. the heterozygous and light males had higher Q_{O_2} values than females but the reverse situation was observed in the dark forms.

Pupal Respiration.—Oxygen uptake rates of one-day- and four-day-old pupae are reported in Table IV. No differences were observed when comparing one-day-old pupae of different strains. Q_{10} values showed no significant tendency for ex-

treme metabolic adaptation (Table II). The metabolic rate at 35°C. was slightly lower than at 30°C.

The metabolic response of four-day-old pupae to temperature differs in certain aspects from that of one-day pupae. A significant strain difference is noted: the dark strain has a lower rate of oxygen uptake than either of the other two types when determined at 25°C. In this respect the late pupae resemble more typically the metabolic behavior of adults than that of the early stage pupae. An increase of temperature from 30° to 35°C. markedly increased the oxygen consumption

TABLE III

Oxygen consumption of males and females of various color strains of Drosophila melanogaster

The figure in the column headed "No." indicates the number of determinations

Temp. °C.	Dark				Heterozygous				Light			
	Males		Females		Males		Females		Males		Females	
	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.
13	12.36	5	12.91	3	16.78	5	15.01	4	16.60	4	13.11	4
18	25.37	5	29.17	5	47.01	5	48.88	5	32.37	5	29.39	5
22	22.38	6	25.84	6	45.01	6	48.08	6	23.52	5	25.51	6
27	43.59	6	45.33	6	54.08	4	65.74	4	50.49	6	51.00	6
30	39.35	6	46.79	6	67.61	5	53.37	4	56.62	6	54.94	6
33	61.36	4	58.47	4	76.56	4	82.24	3	83.05	4	73.94	4
36	80.21	8	62.63	8	83.78	7	66.27	5	80.81	8	75.83	8
39	128.87	4	114.14	4	34.36	4	37.48	5	125.47	4	121.33	4

TABLE IV

Rate of oxygen consumption of various color strains of pupal Drosophila melanogaster *at different temperatures*

Temp. °C.	Dark Strain			Heterozygotes (d/l)			Light Strain		
	No.	Mean Q_{O_2}	S.E.[1]	No.[1]	Mean Q_{O_2}	S.E.[2]	No.[1]	Mean Q_{O_2}	S.E.[2]
One-day pupae									
13	6	3.389	0.837	6	2.777	0.208	4	3.652	0.940
25	10	8.530	0.667	8	8.540	0.580	9	7.663	0.630
30	12	16.881	1.080	11	15.600	1.010	11	16.906	0.724
35	14	12.822	1.025	12	11.760	0.941	9	12.822	1.740
Four-day pupae									
13	4	2.493	0.088	4	2.854	0.404	4	2.653	0.234
25	7	9.199	0.450	7	15.554	0.415	7	13.821	1.180
30	7	16.638	0.420	7	13.364	0.165	7	15.768	0.850
35	7	17.537	1.250	7	24.758	1.030	7	18.031	0.590

[1] Number of determinations

[2] Standard error

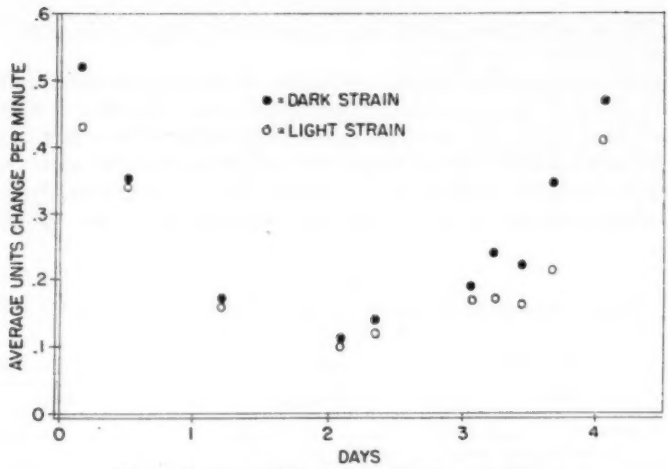

FIG. 1. Oxygen uptake during pupal development

rate of the heterozygotes, whereas the rates for dark and light flies were only slightly higher.

The metabolic rate at 25°C. of light and dark pupae was determined continuously over the duration of the pupal stage. Results are represented in Figure 1. Each point represents the average number of manometer units per minute of eight continuous determinations. Absolute metabolic rate is not represented on this figure but rather the relative increase or decrease in oxygen utilization. In order to determine the absolute metabolic rate, one would have to multiply the number of manometer units used by the flask constant and then divide by the weight of the animal.

Humidity Effects on Metabolism.—Conditions of low relative humidity resulted in higher rates of oxygen uptake than when determined under experimental conditions of high humidity (Table V). Strain and age differences were noted. At 22°C., low humidity did not increase the rate of oxygen uptake of adult heterozygotes, but at 27°C. a pronounced effect was observed. Adult dark and light flies responded similarly. An unusual response was noted in that at 33°C. dark and light adults consumed less oxygen than at 30°C. One-day and four-day pupae differed mainly in their response at 30°C. The four-day pupae responded more like the adults than earlier stages.

Weight.—Results of oxygen consumption measurements reported above were based on the wet weight of the organism. It has been pointed out by various workers that comparative oxygen uptake rates are more meaningful if expressed per unit of dry weight. Data on the wet and dry weights of flies of different strains according to age and sex are reported in Table VI. It is interesting to note that the heterozygous pupae have a slightly higher water content than both the light and the dark forms. However, the opposite relationship is observed in the adult stage.

TABLE V

Rate of ozygen uptake of various color strains of Drosophila melanogaster *when exposed to low relative humidity*

The figure in the column headed "No." indicates the number of determinations

Temp. °C.	Dark		Heterozygous		Light	
	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}	No.	Mean Q_{O_2}
			Adults			
22	8	70.11	9	47.49	8	66.22
27	8	65.70	8	100.77	8	69.42
30	8	124.79	7	105.20	8	129.58
33	12	71.40	8	121.05	11	84.74
			One-day pupae			
25	4	11.718	6	20.613	5	18.440
30	5	15.483	6	15.067	4	14.731
			Four-day pupae			
25	3	13.054	3	14.467	3	13.347
30	3	30.854	3	38.071	3	35.569

When the metabolic rate is based on dry weight, the actual values naturally are different from those based on wet weight, but the relationship of the comparative figures is the same. For example, at 25°C. the oxygen consumption of one-day pupae expressed as ul. of oxygen/minute/mg. (wet weight) for dark, heterozygous, and light is 8.53, 8.54, and 7.66 respectively. These same values expressed as ul./minute/mg. (dry weight) are 33.6, 30.5, and 25.5. It should be pointed out that the greatest variation in water content occurs at this stage of development. Although it did not seem necessary in these experiments to use dry weights in determining metabolic rate, this factor should be kept in mind as a variable in comparative studies.

As demonstrated in other studies on holometabolous insects, the weight decreased during pupal development. Adults weighed less than late stage pupae, and also distinct sexual differences were noted, females weighing more than males. Marked inter-strain differences were found only in males; dark males were heavier than either light or heterozygous males.

DISCUSSION.—Results of the present paper indicate that metabolic differences exist in the color strains of *Drosophila melanogaster* isolated from a single wild population. The metabolic temperature curve of the heterozygous animals was typical of a cold-adapted form when compared with the curves for dark and light forms. Characteristically, a cold-adapted animal will reach a maximum rate of oxygen uptake at a lower thermal level than a warm-adapted form. Thus the metabolic-temperature curve is shifted to the left in a cold-adapted form. Also, the high lethal temperature of the cold-adapted forms is usually lower than

TABLE VI

Weights of various color strains of Drosophila melanogaster *of different age and sex*

	Wet Weight		Dry Weight		Water
	No. of animals	Mean weight (mg)	No. of animals	Mean weight (mg)	(Per cent)
One-day pupae					
Dark	535	1.400	60	0.488	69.68
Heterozygous	525	1.450	20	0.440	72.10
Light	435	1.351	80	0.408	70.02
Four-day pupae					
Dark	405	1.246	45	0.387	69.94
Heterozygous	230	1.225	40	0.356	71.00
Light	405	1.251	43	0.385	69.30
Adults					
Dark males	160	0.945	29	0.252	73.26
Dark females	155	1.147	76	0.330	73.11
Heterozygous males	180	0.868	55	0.234	73.00
Heterozygous females	130	1.157	62	0.340	72.58
Light males	70	0.871	30	0.235	73.89
Light females	70	1.141	43	0.283	73.53

warm-adapted forms. In the present paper, heterozygotes died at a lower temperature than either of the other two forms.

Although the metabolic rates of the dark and light forms were similar at the two temperature extremes, a significant physiological difference was observed at intermediate temperatures (27°–33°C.). The light forms had the higher metabolic rate, a condition normally associated with a more active organism. This physiological difference takes on added significance in the light of the findings of Jacobs (1956), who, working on these same strains of flies, found that dark males departed from light males, when attacked by them, more often than the converse was observed.

Orr (1937) reported interstrain differences in *melanogaster* in that vestigial winged adults consumed more oxygen than the wild type, which in turn had a higher metabolic rate than wild vestigial hybrids. Determinations were made at only one temperature level (25°C.).

An explanation of the mechanism effecting the marked variation in metabolic rates of light and dark strains at the 27°–33°C. interval is difficult to approach. One may assume that various temperatures to which the flies are subjected have a differential effect on over-all metabolism of the two strains. As a result, there arises the problem of temperature relationships of biological activity, i.e. its effect on the over-all reaction rate of biological processes. The problem of mechanisms responsible for change in rate at intermediate temperatures, however, is one of some controversy. Johnson, Eyring, and Polissar (1954) state that with respect to the biochemical activity of a biological system, it is likely that the over-all process is limited largely by one reaction. They also state that the so-called "temperature characteristics" of normally limiting reactions are subject

to variation as a result of differences of chemical environments, such as the presence of inhibitors. It is noted that variations could result from a change in the limiting reaction in a catenary series or from the presence of substances affecting the catalytic activity of the same system.

On the basis of the above statements, it would seem reasonable to assume that the obvious difference in pigmentation between the two strains may indicate the cause of metabolic differences. A limiting reaction may exist which, if altered, could affect over-all metabolism. One need not assume that the process involving the formation of pigment is actually a limiting reaction. It is possible, however, that the equilibrium of the limiting reaction, whatever it is, is dependent on a reaction or reactions involving metabolites concerned in pigment formation. Consequently, variation in melanin formation, for example by an inhibitor affecting enzymatic activity of the process, could be a deciding factor in the organism's metabolism. With a change in temperature, it seems that the limiting reaction in the dark and light strains could cause a change in the difference in metabolic rate as a result of a differential influence on its reaction equilibrium. Some evidence supporting this viewpoint was reported by Ephrussi and Herold (1945), who observed variations in the amounts of red and brown pigments of the eye produced in mutant strains of *Drosophila* when raised at different temperatures.

Metabolic rate determinations made at a number of temperature levels give a better insight into the influence of temperature on metabolism than generalizations from a few widely separated thermal points. Table II shows that when the temperature range is from eight to nine degrees most of the Q_{10} values are close to the typical values of 2–3, and little apparent metabolic adaptation has taken place. However, when the range of the thermal increment is from three to five degrees, marked differences in response to various temperature levels are observed. Certain points along the temperature gradient appear to be of a more "critical" nature for the organism, and this "critical" temperature varies from strain to strain. For example, all three types exhibited a marked increase in metabolic rate when the temperature was raised from 13° to 18°C.: the heterozygote with a Q_{10} of 8.98 was influenced more than the other two strains. However, an additional increase in temperature of 4°C. from 18°–22°C. did not increase the metabolic rate, but resulted in slightly lower values. This response appears to be very much like one obtained by Woodworth (1936) who worked with honeybees. Bees had a higher metabolic rate at 15°C. and 30°C. than at 20° and 25°C. It appeared that these animals had a "zone of comfort" between 20°–25°C. and immediately beyond these extremes the metabolic rate increased. He also observed that at 10°C. the bees were numbed and therefore the respiratory rate was decreased.

In the present investigation it was observed that two leveling-off plateaus existed, 18°–22°C. and 27°–30°C. This is rather interesting in that both of these plateaus are immediately on either side of the temperature at which these animals had been reared (25°C.). It is possible that there is a relationship between this acclimatization temperature and the response of these organisms. Recently

Dehnel and Segal (1956) reported that metabolic compensation to temperature was observed in the American cockroach.

In 1935 Dobzhansky and Poulson measured the rate of oxygen uptake at 14°C. and 25°C. of pupae of two sibling species of *Drosophila*, *pseudobscura* and *persimilis*. Only at the higher temperature were differences observed between the two species. The species which consumed the greater amount of oxygen was normally found in colder areas and this metabolic response was thought to be adaptive. The results of the present paper represent an example of intraspecific differences in *melanogaster*, which may be under control of a single genetic locus.

The present study demonstrates that the metabolic response of four-day pupae is more nearly like that of the teneral adult than of the one-day pupae. Interestingly, early pupae of both strains responded similarily whereas marked differences between the strains were noted in the later stage.

Further differences between early and late pupae are noted in that the early pupae reach a respiratory peak at a lower temperature level than older pupae. This phenomenon of pupal difference is similar to results obtained by Orr (1937), who observed that the initial high oxygen utilization of adults just after emergence corresponds to the high oxygen consumption at the end of the pupal period. It appears that during pupal reorganization the differences in metabolic response noted in these strains become distinctive.

The typical U-shaped curve obtained when the metabolic rate was measured over the entire pupal period is similar to results of other workers (Bodine and Orr, 1925; Dobzhansky and Poulson, 1935; Schweitzer, 1937; Ellenby, 1938; and Birch, 1947).

It should be pointed out that other workers have noted also that a measurable rate of oxygen consumption can be recorded after the death of an organism. Fraenkel and Herford (1940) stated that, for larvae of the blowfly, *Calliphora erythrocephala*, this rate is not always reduced below that of the living state. Birch (1947) found that the insects he was studying maintained a metabolic rate that was at about the maximum value after death. On the other hand, Jefferson (1945) observed that larvae of *Calliphora* and *Sarcophaga* had an increased rate of oxygen consumption after death. He suggested that this response might be correlated with the destruction of the dehydrogenase system.

Previous workers have reported conflicting results in regard to the correlation of sex and rate of oxygen utilization. In this respect no difference between larval males and females of *Drosophila melanogaster* was found by Clare (1925), while Schweitzer (1937) found that female larvae of this same species had a higher metabolic rate than males. Kucera (1934) and Orr (1937) stated that adult female *D. melanogaster* consumed oxygen at a faster rate than males. The opposite results were obtained by Dobzhansky and Poulson (1935) with pupae of *D. pseudobscura*. The findings of the present paper indicate no consistent significant differences between oxygen metabolic rates of males and females over the entire temperature range. A tendency for males to consume more oxygen than females was noted at high non-lethal temperatures.

Although the effects of humidity on respiration may be indirect and represent a complex problem involving many factors (Roeder, 1953), the present study

revealed that the metabolic rate was increased when the organisms were placed in conditions of low humidity. The motility of the adults could not be observed under the present experimental conditions but it is interesting to note that both adults and non-motile pupae responded similarly. Birch (1947) found that humidity levels of 34 per cent and 55 per cent R.H. did not increase the rate of oxygen uptake of *Rhizopertha dominica* and *Calandra oryzae*. The roach, *Blatta orientalis*, which normally prefers dry regions, consumed more oxygen in moist situations than when exposed to lower relative humidity (Gunn and Cosway, 1942). These authors stated that the higher rate in moist air resulted from increased activity and increased body temperature. No differential response by the three types of flies used in this study was noted.

It appears that the heterozygous condition at the ebony locus results in flies which may be better adapted to lower than to higher temperatures. If the degree of oxygen consumption can be employed as an indicator of activity, heterozygotes may have a better chance than the light or dark forms of mating at lower temperatures, but at elevated temperatures the converse would be true. Since little is known of the ecology of *melanogaster* these speculations are tentative. Generally, melanistic forms of insects are found in cool, moist areas and the light forms are collected in hot, arid habitats (Gloger's Rule). If light X dark hybrids are adapted to low temperatures, a system of balanced melanistic polymorphism may be involved at such low temperatures and so would explain the presence of melanistic forms in populations at low temperatures. This would not, however, explain the cases in which an entire low temperature insect population consists of the dark phase as reported, for example, by Dobzhansky (1933) for coccinellid beetles.

SUMMARY

Adaptive physiological responses of melanistic forms of *Drosophila melanogaster* were investigated. From a single wild population, light and dark strains were isolated. The rate of oxygen consumption of light, dark and heterozygous flies was determined at different temperatures and levels of relative humidity. Pupal and adult respiration was studied by manometric techniques.

Metabolic differences exist in the various color strains. Although showing similar responses at extremes of the temperature spectrum, light and dark strains were significantly different at intermediate temperatures. Heterozygotes demonstrated favorable adaptive responses at low temperatures. A possible biochemical mechanism was discussed. When flies were exposed to low relative humidity, the metabolic rate was increased. The response of four-day pupae was more nearly like that of adults than of one-day pupae.

The possible role of heterosis in population composition at reduced temperatures was considered.

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SPONTANEOUS AND COLD-INDUCED HETEROPLOIDY IN *TRITURUS GRANULOSUS*[1]

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INTRODUCTION.—Heteroploidy is a general term used to embrace all degrees of deviation from the normal diploid chromosome number within a species. Heteroploidy may be divided into euploidy, in which the departure from the diploid number is by a multiple of the haploid complement for the particular animal under consideration, and aneuploidy, characterized by the addition or subtraction of one or more chromosomes of the diploid complement. An example of the former is haploidy (N), resulting from a failure of diploid restoration by the normal fertilization process. If a complete haploid set of chromosomes is added to the diploid, the resulting individual possesses three sets and is, therefore, a triploid (3N). All euploid animals with three or more chromosome sets are designated as polyploids (Fankhauser, 1945). Hypo- and hyperdiploidy may be cited as examples of aneuploidy.

In addition to larvae which are completely heteroploid in nature, there are those which show striking variations of chromosome number within a single individual. In some cases the cells differ by multiples of the haploid set. These are known as euploid mosaics. If the cells of an individual differ from each other by single chromosomes rather than by sets, they are called aneuploid mosaics.

Within the last decade increasing interest has been shown in the study of the occurrence of heteroploidy and its morphological and physiological effects as seen in various species of the plant and animal kingdoms. For a detailed presentation of the nature, methods, results to date, and significance of the problem, one may consult the papers by Fankhauser (1939a, 1939c, 1941b, 1942), Fankhauser and Humphrey (1943), Fischberg (1944, 1947a, 1948) and Parmenter (1933), and the comprehensive reviews by Fankhauser (1945, 1955).

MATERIAL AND METHODS.—In the spring of 1942, adult animals of *Triturus granulosus* Skilton (= *T. similans* Twitty as described by Twitty, 1935, 1942) were collected by the senior author from three localities in California: at Saratoga in Santa Clara County; at Ukiah, Mendocino County (along Robinson's Creek); and at Benbow, Humbolt County (along the Eel River). Stebbins (1954) refers to this species as "*Taricha granulosa* (subspecies)." The Saratoga forms were *T. granulosus twittyi* Bishop. The Ukiah and Benbow forms were either *T. granulosus similans* Twitty or *T. granulosus granulosus* Skilton; at that time

[1] The experimental portion of this investigation and the preparation of all tailtips were done by the senior author at Stanford University, in 1942, during the tenure of a Rockefeller fellowship. Grateful acknowledgement is made of the cooperation of Professor V. C. Twitty, who generously made available excellent facilities for the work.

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the sub-species had not been distinguished. Bishop (1943) regards all individuals of *T. granulosus* from Ukiah northward as *T. granulosus granulosus* Skilton; Myers (1942) considers the Ukiah forms to be *T. granulosus similans* Twitty and regards *T. granulosus granulosus* Skilton as a more northerly form. As no cytological differences were noted in the animals from the various localities, they have been considered together in this study.

Eggs were obtained from April 3 to June 27 by means of pituitary stimulation or were spawned naturally in laboratory tanks. *Triturus granulosus* lays its eggs singly on leaves or twigs rather than in masses. Most of the eggs were obtained by stripping the females a few days after pituitary implantation. (By this method, batches of three to five eggs are forced out of the uterus at a time.) The females were also allowed to deposit their eggs; collections of these were made at intervals of a few minutes, hours, or overnight.

In the earlier experiments the stripped eggs were dipped into a fresh sperm suspension. Later it was found that the eggs cleaved without this treatment, indicating that the females had mated prior to pituitary implantation.

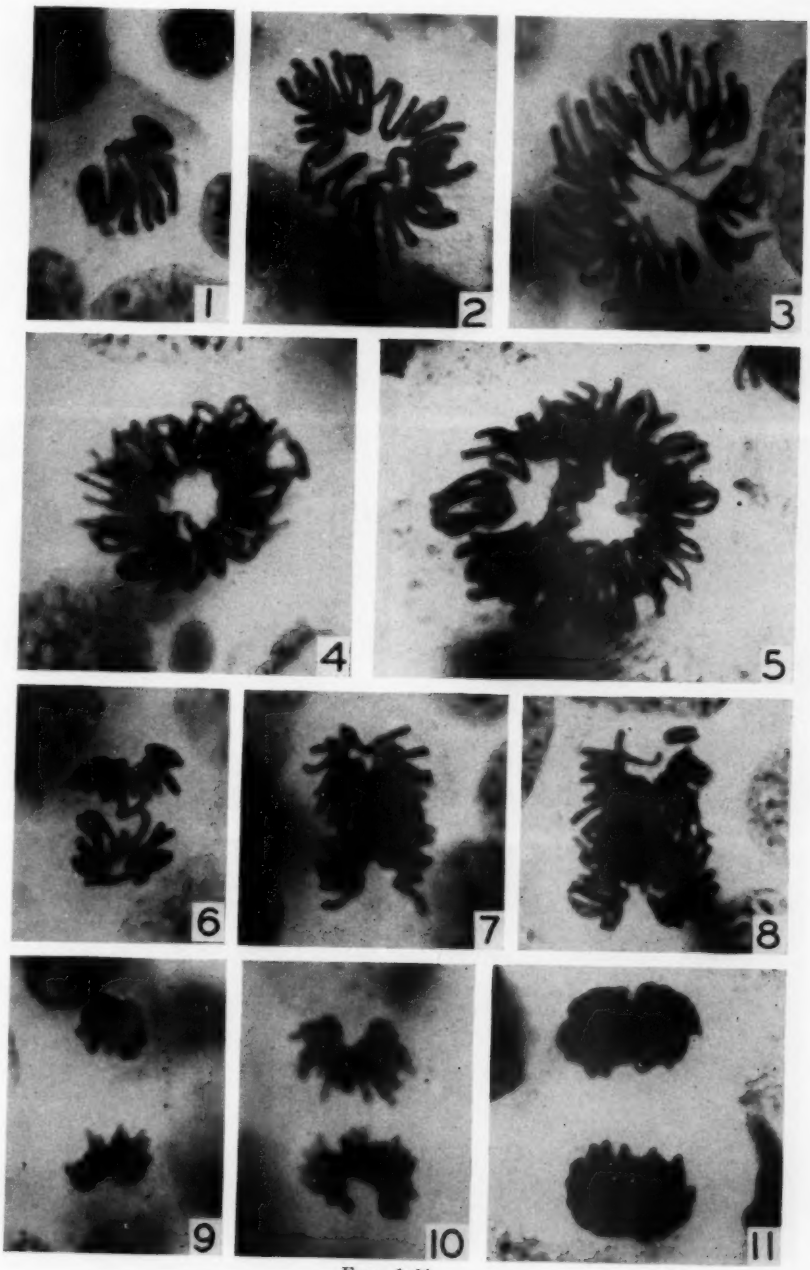
We do not know when the sperm actually enters the egg of *Triturus granulosus* in relation to the time of normal egg deposition, or in relation to the time of artificial insemination.

Following the refrigeration technique of Fankhauser and Griffiths (1939) and Griffiths (1941) for the production of triploids, some of the eggs were collected at various intervals after insemination and placed in the ice compartment of a refrigerator for periods ranging from one hour to several hours. Others were placed in less cold portions of the refrigerator for periods up to four days (Table II). The eggs were then returned to room temperature. Heteroploids were produced in lots treated from 2 to 24¼ hours. This indicates that the duration of the treatment is not of primary importance. The remainder of the eggs were untreated and maintained at room temperature as controls.

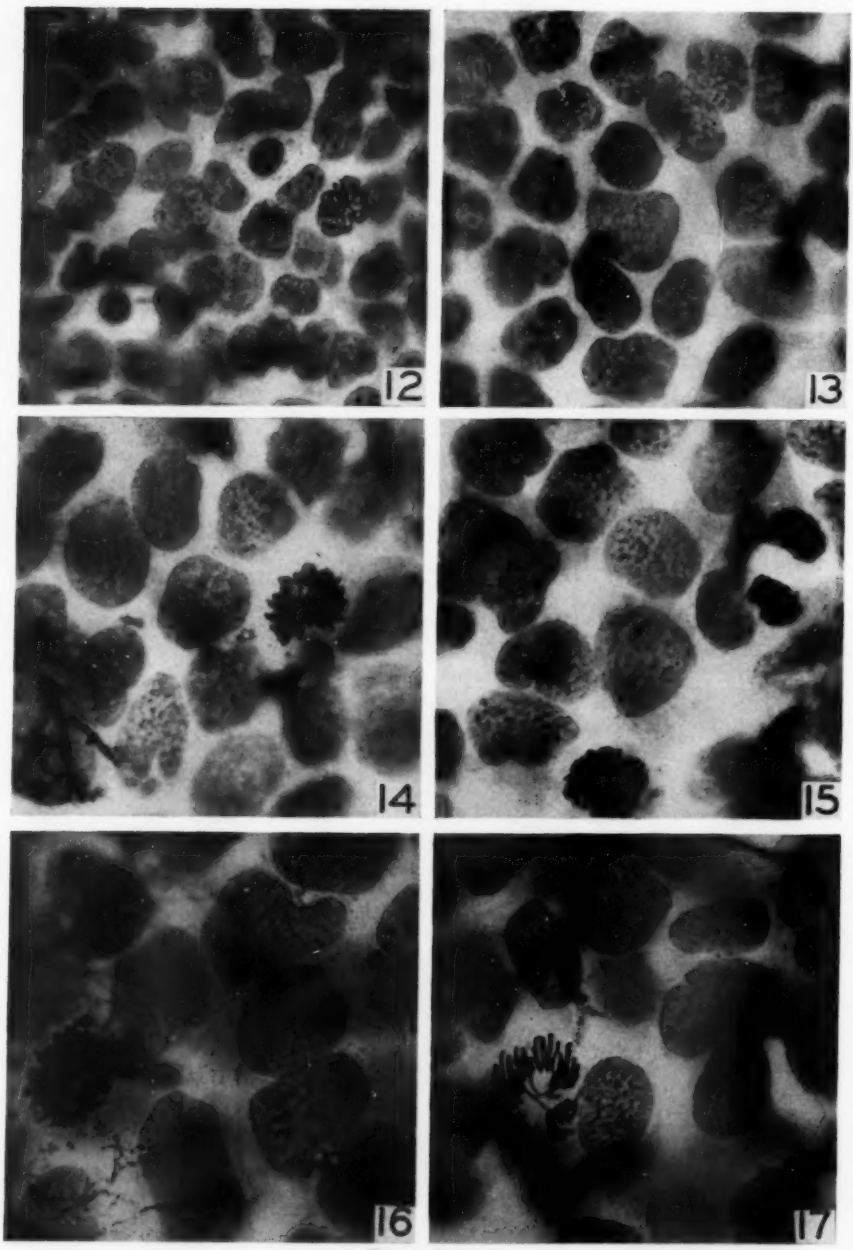
When the experimental and control larvae reached an age of about four weeks (22 to 34 days), the tailtips were clipped, fixed, stained and mounted *in toto* for cytological study. The larvae were permitted to continue development and in some cases a second tailtip was obtained. Tailtips of this species clipped as early as 15 to 17 days after insemination are yolky and worthless as cytological preparations. The rate of yolk absorption undoubtedly varies with the different developmental abnormalities which may appear in some of the experimental animals. All larvae surviving at the end of the experiment were fixed for subsequent study.

Bouin's fixative was used and all tips and sections were stained with Mayer's acid hemalum. Several heteroploid and control larvae were serially sectioned at a thickness of ten micra. The sections were counterstained in a 0.2 per cent solution of Eosin Y in 90 per cent alcohol.

FIG. 1–11. Mitotic figures from tail-tip epithelium of *Triturus granulosus* larvae. ×1125. FIG. 1–5. Haploid, diploid, triploid, tetraploid, pentaploid metaphases, respectively. FIG. 6–8. Haploid, diploid, triploid early anaphases, respectively. FIG. 9–11. Haploid, diploid, triploid late anaphases, respectively.



FIGS. 1–11



FIGS. 12–17

OBSERVATIONS. *General.*—A brief report (Costello, 1942) of the incidence of heteroploidy among 100 larvae developing from cold-treated eggs of *Triturus granulosus* revealed 13 triploids, 81 diploids, four haploids, and two haplo-diploid mosaics. The present report is a continuation of this preliminary study. It is based on further observations on the tailtips of the 100 larvae previously studied, as well as an investigation of tailtips from an additional 28 experimental animals and 184 untreated larvae used as controls. Included in the observations are: (1) a determination of the chromosome number of the species; (2) a comparative study of nuclear and cell sizes in cold-induced and spontaneous heteroploids and diploids; and (3) a report of the frequency of spontaneously occurring heteroploidy based on a study of the control larvae.

Chromosome Number.—Chromosome counts of a large number of metaphase stages in the epidermis of the tailtips of control animals showed the normal diploid chromosome number to be 22. In an effort to identify the individual chromosomes in this species, a few drawings were made arranging them in a series according to the approximate lengths of the arms on either side of the point of spindle fiber attachment. In these mitotic figures the chromosomes do not lie in one plane but must be followed by focusing with the fine adjustment of the microscope (see the paper by Parmenter, 1919). Added to this difficulty in obtaining true measurements was that of the foreshortening of some chromosomes because of the twisting of the chromatids. No corrections were applied for these possible errors, but figures in which each chromosome lay predominantly in one plane were chosen in order to minimize distortion.

The chromosomes of each mitotic figure were arranged linearly according to the length of the arms and formed a series exhibiting a gradual increase in size. Grouping into homologous pairs, as judged by arm length on either side of the spindle fiber attachment point, was possible to some extent, and the chromosomes of one mitotic figure were closely comparable with those of another. The individuality of the chromosomes was further emphasized when a haploid series was studied for comparison with the diploid. It was clear from this comparative study that the haploid chromosome complement consisted of one member of each pair of the diploid chromosomes found in the somatic cells of this species.

Nuclear Size.—By careful comparison of nuclear areas in haploid, diploid, and triploid individuals (diagnosed by chromosome counts), it was apparent that in this species, as in certain others, the nuclear area can be used as a direct indication of chromosome constitution. It is possible to use the area of the optical section at the level of focus of the greatest perimeter of the nuclei for measurements because of the high order of nuclear flattening exhibited. As shown by Fankhauser (1945), the areas of the nuclei increase in approximately the same ratio as the volumes, for, as part of the general regulation toward normal body size, the

FIG. 12–17. Epidermal cell nuclei (with some mitotic figures) from tail-tip epithelium for nuclear size comparisons. ×500. FIG. 12. Haploid no. 166b. FIG. 13. Diploid no. 186. FIG. 14. Triploid no. 140. FIG. 15. Diploid/tetraploid mosaic no. 187. Tetraploid area with tetraploid mitotic figures. FIG. 16. Pentaploid no. 230b. FIG. 17. Triploid/hexaploid mosaic no. 218a. Triploid area with triploid metaphase shown also in figure 3.

TABLE I

Summary of chromosome condition of cold-treated and control larvae of Triturus granulosus

	Number of Larvae	Diploid 2N	Haploid N	Triploid 3N	Tetraploid 4N	Pentaploid 5N	Others
Control	184	180	0	0	0	1	2(2N/4N) 1(3N/6N)
Experimental	128	98	7	17	1	0	3(2N/4N) 2(N/2N)

thickness of the nuclei remains almost constant from the haploid to the tetraploid level of chromosome constitution.

Drawings for comparison of nuclear sizes were made with the aid of a camera lucida at a magnification of seventeen hundred diameters. All complete nuclei whose projected images were enclosed within the limits of standard 5″ x 8″ cards were traced. In this manner, comparable areas of all tailtips were drawn, and both nuclear and cell sizes, as indicated by the spacing of the nuclei, were studied.

Heteroploidy. —A summary of the incidence of heteroploidy among all experimental and control larvae, based on studies of nuclear size and actual chromosome counts, is given in Table I. The eggs were collected over intervals of a few minutes to a few hours. The ages indicated at the onset of treatment are minimum times after laying.

More than 703 eggs were subjected to cold, but this number is not very significant, since it is not certain that all were fertilized. Of the experimental eggs, 451 were alive when examined one to three days after treatment. Of this number only 128 survived to the time of tailclipping or an age of about four weeks. This high mortality rate (71.7%) can be correlated with the extreme temperature and long duration of exposure of the eggs. More significant figures are obtained when the data are summarized in intervals of increasing length of treatment (Table II). Thus, it is seen that of the eggs treated only 1¾ hours, 40 per cent survived, giving a mortality rate of 60 per cent which is considerably lower than that indicated for the entire group. On the other hand, from 114 eggs treated at intervals varying from 23 hours to 4 days, only eight lived to the age of tailclipping (93% mortality). This indicates a higher mortality with increased duration of treatment.

Induced Triploidy.—Of the 128 experimental animals, 98 (76.6%) were unaffected by the cold treatment and developed as normal diploids. Seventeen (13.3%) were triploids, with 33 chromosomes. The percentage of triploids produced by these experiments is low compared with the effectiveness of cold shock in producing triploids in other species. This is undoubtedly due, at least in part, to the fact that eggs were treated at varying times after laying. Some, after overnight collection, had already begun cleavage. However, it is interesting to note that many of the triploids produced were from batches of eggs where treatment was begun 75 minutes or longer after egg deposition (Table III). Fankhauser and Griffiths (1939) obtained a high percentage of triploids only

TABLE II

Summary of cold treatments

Length of Treatment	Number Treated	Survived to Tail-clipping	Percent Surviving	Mortality Rate (%)
1 to 3¾ hrs.	135	54	40.0	60.0
4 to 5⅔ hrs.	126	12	9.5	90.5
6 to 9 hrs.	70	20	29.0	73.0
15 to 18 hrs.	160	18	11.0	89.0
19 to 22 hrs.	106	16	15.0	85.0
23¼ hrs. to 4 days	114	8	7.0	93.0

when the eggs of *T. viridescens* were treated a few minutes after fertilization. If refrigeration was delayed for thirty minutes, cold was no longer effective, since the second maturation division had progressed to anaphase by this time (Fankhauser and Moore, 1941a). The results for *T. granulosus* may indicate the existence of more than one cytological mechanism responsible for the production of triploidy.

There was an increase in nuclear and cell size, accompanying the increase in chromosome number in the triploids, as seen by measurements of nuclei from tailtip preparations and by observations of the size and number of pigment cells in the living animals. Regulation for the maintenance of normal body size by decrease in cell number is made evident by the fact that 13 of the 17 triploid larvae were normal in gross appearance and developmental rate and fell within the diploid range of body size.

Four triploid animals showed various abnormalities and lagged in development. These four were dwarfed and oedematous. Two were microcephalic and abnormal in the branchial cartilages, and one was completely lacking in external gills.

The salamander egg is arrested at the metaphase of the second maturation division until sperm entry. Following fertilization of *Triturus viridescens* eggs (Fankhauser and Moore, 1941a), the division progresses to anaphase at approximately thirty minutes after insemination and is completed with the formation of the second polar body at about one hour. It has been postulated (Fankhauser and Griffiths, 1939) that temperature shock (either extreme cold or heat) immediately after egg-laying suppresses the second maturation division and causes the retention within the egg of the chromosomes of the second polar body. Furthermore, it has been assumed that the chromosomes of the egg and polar body form a diploid restitution nucleus. The fusion of such a nucleus with the haploid sperm nucleus would result in the production of a triploid individual.

Briggs (1947) produced triploids in *Rana pipiens* by exposing the eggs 20 minutes after insemination to a temperature of 37° C. for four minutes. In Briggs' experiments an average of 38 per cent of all eggs treated developed into normal triploids. By closely observing a group of treated eggs during very early development, he found that no second polar body was given off in the eggs which later developed as triploids.

TABLE III

Summary of results of cold treatment experiments

Animal	Lot	Hours Treated	Conditions	Age at Treatment	Days Survival
			Triploid		
140	May 26, 11:10	5½	Ice compartment frozen	35 min. after insemination	44– (died)
141b	May 26, 11:10	5½	Ice compartment frozen	"	39– (died)
143	May 26, 2:50	17	Ice compartment 1 hr. Refr. 16 hrs. Not frozen	80 min. after insemination	39– (died)
149b	May 26, 2:50	17	"	"	90 (fixed)
152	May 26, 2:50	17	"	"	89 (fixed)
153	May 26, 2:50	17	"	"	89 (fixed)
155a	May 26, 1:30	2½	Ice comp. 2½ hrs. frozen	75 min. after insemination	90? (fixed)
159	May 26, 1:30	2½	"	"	44– (died)
161	May 26, 1:30	2½	"	"	84
162	May 26, 1:30	2½	"	"	39– (died)
166b	May 26, 1:30	2½	"	"	35–44 (died)
172	May 27, 10:40	5	Ice comp. 5 hrs. frozen	50 min. after insemination	88 (fixed)
193a	May 27, 9:25	6	Ice comp. 6 hrs. frozen hard	65 min. after insemination	42– (died)
239	June 10, 3:40	19	Not frozen	Eggs collected over 6 hr. period	75 (fixed)
244	June 13, 10:05	8¼	Ice comp. 1½ hrs. Not frozen	Uncleaved eggs coll. overnight, some stripped	26– (died)
261	June 14, 10:10	23¼	Ice comp. 1½ hrs. Not frozen	Collected overnight, uncleaved eggs	45– (died)
262	June 14, 10:20	23¼	Ice comp. 1½ hrs. Not frozen	Freshly laid, not artificially inseminated	71 (died)
			Tetraploid		
4	April 9, 1:45	20½	Ice comp. 2 hrs. frozen, then refr. 18 hrs.	36 min. after insemination	47 (fixed)
			Haploid		
2	April 3, 2:50	19	Ice comp. 1½ hrs., frozen	20 min. after insemination	146 (fixed)
8	April 3, 2:50	19	"	"	34+ (died)
6	April 8, 2:10	21½	Ice comp. 1 hr.	35 min. after insemination	139+ (fixed)
141a	May 26, 11:10	5½	Ice comp. 5½ hrs., frozen	"	44– (died)

TABLE III. *Continued*

Animal	Lot	Hours Treated	Conditions	Age at Treatment	Days Survival
			Haploid (cont'd)		
166a	May 26, 1:30	2½	Ice comp. 2½ hrs., frozen	75 min. after insemination	35–44 (died)
196b	May 26, 11:00	3¾	Ice comp. 3¾ hrs.	Collected overnight	48–56 (died)
220	June 5, 3:10	2	Refr. 2 hrs., not frozen	Collected over 2 hrs. stripped 10 min. after insemination	32 (died)
			Haplo-Diploid Mosaics		
223	June 9, 9:12	24¼	Refr. 24¼ hrs. frozen	Collected overnight	23– (died)
224	June 9, 9:12	24¼	"	"	70 (fixed)
			Diplo-Tetraploid Mosaics		
182	May 26, 11:00	3¾	Ice comp. 3¾	75 min. after insemination	91 (fixed)
187	May 26, 11:00	3¾	"	"	90 (fixed)
255	June 13, 10:05	8¼	Ice comp. 1½ hrs. (not frozen)	Uncleaved eggs collected overnight plus some stripped	46– (died)

At the onset of heat treatment in Briggs' experiments the *Rana* eggs were at the beginning of the anaphase separation of the chromosomes. Briggs proposes that the primary effect of temperature shock is an inhibition of the shortening of the chromosomal spindle fibers which occurs in the first phase in the movement of the chromosomes from the metaphase plate. It is at the approximate time when this would occur that the *Rana pipiens* eggs were most sensitive to the heat treatment.

Direct evidence for the formation of a diploid egg nucleus by the suppression of the second maturation division in refrigerated eggs of the carp, *Cyprinus carpio*, has been presented by Makino and Ozima (1943). In the carp, as in the salamander, the egg at the time of laying is in the metaphase of the second maturation division and further progress of mitosis is dependent upon the entrance of the sperm. Fertilized eggs were refrigerated at temperatures ranging from 0.5° C. to 3.0° C. for ten, twenty, and thirty minutes. Cold treatment was begun ten minutes after insemination, at which time the maturation division has progressed to anaphase. Microscopical examination of these eggs in section gave evidence that after cold treatment, mitosis was arrested in anaphase and the second polar body was never given off. Furthermore, a nuclear membrane developed surrounding the daughter chromosomes, and a diploid nucleus was formed by the retention of the chromosomes which would normally have been eliminated with the formation of a second polar body. The subsequent union of

such a diploid nucleus with the sperm nucleus would result in the formation of a triploid individual.

An investigation of the mechanism of triploid production by heat treatment of the eggs of *Triturus viridescens* has also been made (Fankhauser and Godwin, 1948). Microscopical examination of sections of 22 of a group of 45 eggs fixed at various intervals after heat treatment reveals that in this species the primary effect of the heat is not the disruption of the spindle mechanism. Instead, it is the submergence of the second maturation spindle below the surface of the egg, with a continuation of the chromosomal division and the subsequent formation of two haploid egg nuclei. These egg nuclei may be distinguished easily from the differentiating sperm nuclei which are accompanied by conspicuous asters. The fate of the two egg nuclei has been ascertained. The union of the egg and sperm nuclei normally occurs approximately four hours after egg-laying. In three heat-treated eggs fixed at this stage, two egg nuclei may be seen in contact with a sperm nucleus between the two asters formed by the division of the sperm centrosome. Indication that further development in the eggs is normal is found in an egg fixed 5½ hours after treatment, in which there is present a normal anaphase of the first cleavage mitosis having 33 chromosomes, the triploid number, at each pole.

Of the 45 experimental eggs examined (22 were sectioned) by Fankhauser and Godwin, only three gave any evidence of the formation of a diploid egg nucleus. Two showed abortive anaphases displaced below the surface of the egg in which the chromosome groups were very close together. The third contained a single egg nucleus considerably larger than those in the controls of the same age. Thus, it appears that there is some variation in response to the heat treatment in eggs of a single species. It is possible, therefore, that other mechanisms may be involved in triploidy-induction by refrigeration and in the response to temperature shock, both heat and cold, in other species.

Our results may be compared with those of Böök (1940), Fankhauser (1938c, 1939b, 1940a), Fankhauser, Crotta and Perrot (1942), Fankhauser and Humphrey (1942), Fankhauser and Watson (1942) and Fischberg (1945).

Induced Tetraploidy.—A single tetraploid individual was found among the 128 experimental larvae. This tetraploid (No. 4, April 9th, cold-treated) was from a batch of eggs treated 36 minutes after deposition. The eggs were placed in the ice compartment for two hours. On removal it was noted that these eggs had been frozen. They were then placed on the refrigerator shelf at a higher temperature for 18 hours. The tetraploid condition may be accounted for by chromosome duplication during the first cleavage occurring during the long refrigeration period. Another possible, but less likely, explanation would be the chance union of two diploid gametes.

The tetraploid was markedly dwarfed with a striking pigment pattern of few but very large chromatophores. It could not be induced to feed after the exhaustion of its yolk supply. During several trials, spaced over several days, it showed no feeding response to *Cladocera*. The larva was preserved at the age of 47 days when it began to show signs of starvation. Two tailtips and serial sections of the

animals were studied. In gross and general microscopic anatomy, the tetraploid appeared quite normal; however, nuclear and cell sizes were approximately twice those of diploids of the same age.

Only three tetraploid salamander larvae have been described in the literature to date.

Induced Haploidy.—Of 128 experimental larvae seven (5.4%) were haploid, and of the seven haploids, four were from lots in which the eggs were actually frozen in the ice compartment. The haploids were easily discernible among the larvae by their external appearance. They were dwarfed, lagged in development, and contained a larger number of cells per unit area than the diploids, as indicated by the pigment pattern. None of these larvae exhibited such abnormalities as ascites and microcephaly which are frequently characteristic of haploids of other species. Six were quite normal in appearance, although small in size, and the seventh showed only a twisted spine in the tail region. Nevertheless, five of the animals died at ages between 4½ and 8 weeks. Two (No. 2, April 3, and No. 6, April 8) were fixed in good condition at the ages of 146 and 139 days, respectively, when the experiment was terminated.

A detailed study was made of the oldest haploid (No. 2, April 3). Although relatively normal in appearance, this animal lagged perceptibly in development. In a photograph taken 33 days after the cold treatment, the haploid is seen to be slightly smaller than a diploid of the same age and to possess more pigment cells per unit area. A photograph taken two months later of the same animals illustrates even more strikingly the differences in size and developmental rate. Whereas the diploid had completed metamorphosis, the haploid possessed long external gills and a prominent dorsal fin. The haploid was still in a larval state at fixation. At this time it was 32.8 mm. in length as compared with the diploid control length of 45 mm. The animal was normal in appearance except for the pigment pattern and a slightly twisted spine.

This haploid and a diploid control were serially sectioned. Chromosome counts from 50 metaphase figures distributed throughout the body of the experimental larva verified the fact that it was haploid (11 chromosomes) throughout. The tissue distribution of these chromosome counts was as follows: cartilage, 5; connective tissue, 9; cornea, 2; endothelium of blood vessels, 1; gill epithelium, 1; nasal epithelium, 4; oral epithelium, 1; epidermis, 18; liver, 6; spinal cord, 2; telencephalon, 1. In addition, drawings of nuclei from comparable regions of the telencephalons, innermost layers of the retinae, and from Harder's glands indicate an approximate ratio of one to two in nuclear size between the haploid and diploid.

A summary of the results of refrigeration experiments in four other species of salamanders (Fankhauser, 1945, p. 40) shows a low percentage of haploids produced in each species. This summary is based upon the work of Fankhauser (1937, 1938a, 1938b), Fankhauser and Moore (1941b), Fischberg (1947b, 1947c), Parmenter (1940) and Porter (1939), in addition to those mentioned below. In the majority of cases it is not known whether these individuals developed gynogenetically or androgenetically. One haploid axolotl larva which developed from

such a diploid nucleus with the sperm nucleus would result in the formation of a triploid individual.

An investigation of the mechanism of triploid production by heat treatment of the eggs of *Triturus viridescens* has also been made (Fankhauser and Godwin, 1948). Microscopical examination of sections of 22 of a group of 45 eggs fixed at various intervals after heat treatment reveals that in this species the primary effect of the heat is not the disruption of the spindle mechanism. Instead, it is the submergence of the second maturation spindle below the surface of the egg, with a continuation of the chromosomal division and the subsequent formation of two haploid egg nuclei. These egg nuclei may be distinguished easily from the differentiating sperm nuclei which are accompanied by conspicuous asters. The fate of the two egg nuclei has been ascertained. The union of the egg and sperm nuclei normally occurs approximately four hours after egg-laying. In three heat-treated eggs fixed at this stage, two egg nuclei may be seen in contact with a sperm nucleus between the two asters formed by the division of the sperm centrosome. Indication that further development in the eggs is normal is found in an egg fixed 5½ hours after treatment, in which there is present a normal anaphase of the first cleavage mitosis having 33 chromosomes, the triploid number, at each pole.

Of the 45 experimental eggs examined (22 were sectioned) by Fankhauser and Godwin, only three gave any evidence of the formation of a diploid egg nucleus. Two showed abortive anaphases displaced below the surface of the egg in which the chromosome groups were very close together. The third contained a single egg nucleus considerably larger than those in the controls of the same age. Thus, it appears that there is some variation in response to the heat treatment in eggs of a single species. It is possible, therefore, that other mechanisms may be involved in triploidy-induction by refrigeration and in the response to temperature shock, both heat and cold, in other species.

Our results may be compared with those of Böök (1940), Fankhauser (1938c, 1939b, 1940a), Fankhauser, Crotta and Perrot (1942), Fankhauser and Humphrey (1942), Fankhauser and Watson (1942) and Fischberg (1945).

Induced Tetraploidy.—A single tetraploid individual was found among the 128 experimental larvae. This tetraploid (No. 4, April 9th, cold-treated) was from a batch of eggs treated 36 minutes after deposition. The eggs were placed in the ice compartment for two hours. On removal it was noted that these eggs had been frozen. They were then placed on the refrigerator shelf at a higher temperature for 18 hours. The tetraploid condition may be accounted for by chromosome duplication during the first cleavage occurring during the long refrigeration period. Another possible, but less likely, explanation would be the chance union of two diploid gametes.

The tetraploid was markedly dwarfed with a striking pigment pattern of few but very large chromatophores. It could not be induced to feed after the exhaustion of its yolk supply. During several trials, spaced over several days, it showed no feeding response to *Cladocera*. The larva was preserved at the age of 47 days when it began to show signs of starvation. Two tailtips and serial sections of the

animals were studied. In gross and general microscopic anatomy, the tetraploid appeared quite normal; however, nuclear and cell sizes were approximately twice those of diploids of the same age.

Only three tetraploid salamander larvae have been described in the literature to date.

Induced Haploidy.—Of 128 experimental larvae seven (5.4%) were haploid, and of the seven haploids, four were from lots in which the eggs were actually frozen in the ice compartment. The haploids were easily discernible among the larvae by their external appearance. They were dwarfed, lagged in development, and contained a larger number of cells per unit area than the diploids, as indicated by the pigment pattern. None of these larvae exhibited such abnormalities as ascites and microcephaly which are frequently characteristic of haploids of other species. Six were quite normal in appearance, although small in size, and the seventh showed only a twisted spine in the tail region. Nevertheless, five of the animals died at ages between 4½ and 8 weeks. Two (No. 2, April 3, and No. 6, April 8) were fixed in good condition at the ages of 146 and 139 days, respectively, when the experiment was terminated.

A detailed study was made of the oldest haploid (No. 2, April 3). Although relatively normal in appearance, this animal lagged perceptibly in development. In a photograph taken 33 days after the cold treatment, the haploid is seen to be slightly smaller than a diploid of the same age and to possess more pigment cells per unit area. A photograph taken two months later of the same animals illustrates even more strikingly the differences in size and developmental rate. Whereas the diploid had completed metamorphosis, the haploid possessed long external gills and a prominent dorsal fin. The haploid was still in a larval state at fixation. At this time it was 32.8 mm. in length as compared with the diploid control length of 45 mm. The animal was normal in appearance except for the pigment pattern and a slightly twisted spine.

This haploid and a diploid control were serially sectioned. Chromosome counts from 50 metaphase figures distributed throughout the body of the experimental larva verified the fact that it was haploid (11 chromosomes) throughout. The tissue distribution of these chromosome counts was as follows: cartilage, 5; connective tissue, 9; cornea, 2; endothelium of blood vessels, 1; gill epithelium, 1; nasal epithelium, 4; oral epithelium, 1; epidermis, 18; liver, 6; spinal cord, 2; telencephalon, 1. In addition, drawings of nuclei from comparable regions of the telencephalons, innermost layers of the retinae, and from Harder's glands indicate an approximate ratio of one to two in nuclear size between the haploid and diploid.

A summary of the results of refrigeration experiments in four other species of salamanders (Fankhauser, 1945, p. 40) shows a low percentage of haploids produced in each species. This summary is based upon the work of Fankhauser (1937, 1938a, 1938b), Fankhauser and Moore (1941b), Fischberg (1947b, 1947c), Parmenter (1940) and Porter (1939), in addition to those mentioned below. In the majority of cases it is not known whether these individuals developed gynogenetically or androgenetically. One haploid axolotl larva which developed from

a spawning by a recessive white female mated with a heterozygous dark male showed a characteristic dark pigment pattern thus revealing its androgenetic origin (Humphrey and Fankhauser, unpublished results; see the review of Fankhauser, 1945).

Further evidence of the androgenetic origin of haploids was suggested by the results of refrigeration of *Triturus rivularis* eggs inseminated with *Triturus torosus* sperm (Costello, 1942). Of the twenty larvae obtained, two possessed accentuated paternal characteristics. They had typical haploid abnormalities but the nuclear size was that of the hybrids. It was suggested in that abstract that the cold treatment completely destroyed the second maturation mitotic mechanism, the embryos developing from the division of the foreign sperm nucleus with a duplication of the haploid chromosome complement.

Work by Rostand (1933, 1934, 1936) has given evidence that haploid larvae may arise gynogenetically from refrigerated anuran eggs. Eggs of *Bufo* and *Hyla* inseminated by *Rana* sperm do not develop beyond the blastula stage. However, if the eggs are refrigerated immediately after insemination some of the offspring survive to the larval period and the majority are haploids. It has been postulated that in such cases the low temperature prevents the fusion of the egg and sperm nuclei, the haploid larvae developing under the influence of the maternal chromosomes alone.

In the present experiments a group of eggs was given what was considered an almost lethal exposure to cold in an attempt to destroy the entire chromosome complement of the egg and produce haploids of androgenetic origin. It was considered that the egg chromosomes at the metaphase of the second maturation division would be more likely to be destroyed by the formation of ice crystals than the more compact sperm nucleus which had entered the egg shortly before the onset of refrigeration.

The development of two of the *Triturus granulosus* haploids to advanced stages indicates their essential normality, and is in harmony with the fact that species hybrids between *T. granulosus*, *T. torosus*, and *T. rivularis* are remarkably viable. In terms of a genetic explanation this may perhaps indicate a low index of recessive lethals or deficiencies in *T. granulosus* and the related species of California salamanders as compared with other salamanders and anurans.

Induced Mosaics.—Among the experimental animals there were five (4%) euploid mosaics. Two of the animals (Numbers 223 and 224) were haplo-diploid, each containing some mitotic figures with eleven chromosomes and others with twenty-two. These larvae were both from a batch of eggs collected overnight and placed in the ice compartment on the morning of June 9th. Some of the eggs were cleaving at that time. The eggs remained in the refrigerator for 24¼ hours and were frozen when removed. Of the group treated, twelve were alive on June 11th, four on June 16th, and three survived to tail-clipping on July 1st. This third animal was a diploid.

Number 223 was dwarfed, microcephalic, and oedematous. This animal died twenty-three days after treatment. Number 224 was normal in body size and appearance, and was fixed at the age of 70 days.

If the effect of cold on the salamander egg is presumed to be the same as heat, the haplo-diploid condition might have arisen by the union of a sperm nucleus with one of the haploid egg nuclei (formed by the division of a submerged second maturation spindle) with the separate division of the other egg nucleus to give rise to haploid generations of cells. Other theoretical possibilities are as follows: (1) the division of the sperm nucleus alone with subsequent chromosome duplication in one cell of the two-cell stage; (2) by dispermy, with union of the egg nucleus and one sperm and separate division of another sperm nucleus; and (3) by division of the sperm nucleus and delayed union of one of the daughter nuclei with the egg nucleus.

Numbers 182, 187, and 255 were diplo-tetraploid mosaics. The first two were derived from the same batch of eggs which were cold-treated for 3¾ hours on May 26th in the ice compartment of the refrigerator. These animals were fixed at ages of 90 and 91 days, respectively. Number 255 was cold-treated for 1½ hours in the ice compartment, but not frozen. It was within the normal range in body size but it, too, contained exceedingly large pigment cells. It died by the forty-sixth day after treatment. All three individuals developed from batches of eggs collected overnight, and it is possible that treatment occurred at a time when development had advanced to early cleavage.

The suppression of complete anaphasic separation of one of the dividing cells of the two-cell stage is a possible explanation if the animals are diplo-tetraploid throughout. However, if the tetraploid cells in the tailtip represent only a polyploid patch of tissue, the chromosome doubling must have occurred at a much later stage of development. The essential normality of the larvae would seem to favor the latter explanation.

Henley (1950) obtained a large number (5%) of chromosomal mosaics by cold treatment of *Triturus torosus* embryos at various times after egg deposition.

Spontaneous Heteroploids.—A study of the tailtips from the 184 untreated control larvae revealed three heteroploids; thus, as in other species of salamanders studied, heteroploidy may occur spontaneously in *Triturus granulosus*. Number 230b, a pentaploid, developed from an untreated egg and lived for 44 days. This animal was relatively normal in appearance but had extremely large chromatophores which were very widely spaced. The epidermis of the tailtip contained many metaphase figures. In all these figures, there were areas in which the chromosomes were so crowded as to prohibit accurate counting. The results of the attempted counts varied from over 46 to over 50 distinguishable chromosomes. In every instance the tetraploid number of chromosomes was exceeded.

To obtain additional evidence of the degree of polyploidy exhibited by this animal, an approximate ratio of nuclear sizes in haploids, diploids, triploids, and tetraploids was obtained in a manner similar to that used earlier by Fankhauser (1941a). Groups of twenty epidermal nuclei from each type were drawn at a magnification of seventeen hundred diameters on the same weight of paper. These were cut out with fine scissors and then each group was weighed. A ratio of 0.8:2.0:2.9:3.8 was obtained for the haploids, diploids, triploids, and tetraploid, respectively. A group of nuclei from animal 230b was similarly weighed and

gave a ratio of 2.0:4.8 with the diploid. This closely approximates the expected ratio of 2:5 between diploids and pentaploids. Spontaneous pentaploidy has been reported in *T. viridescens*, *T. pyrrhogaster*, and the axolotl (Fankhauser, 1945).

Fankhauser (1945) has suggested that pentaploid zygotes originate by the union of a tetraploid gamete with a normal haploid gamete. Such a tetraploid gamete might be formed from a diploid ovum by the suppression of both maturation divisions. Another possibility would be meiosis in a tetraploid cell proceeding normally through the first maturation division with the suppression of second polar body formation.

Costello and Henley (1949) found no polyploids or haploids among 582 larvae in a natural population of *Triturus torosus*. Three mosaics, however, were present. One was diploid/tetraploid, the other two diploid/hyperdiploid.

Spontaneous Mosaicism.—There were two euploid mosaics among the controls. One animal, Number 215, was diplo-tetraploid. Number 215 was slightly smaller than the diploid controls and possessed some very large pigment cells. The second tailtip of Number 215 is almost entirely diploid, but contains a few large cells. The other mosaic, Number 218a, was triplo-hexaploid. It was quite light in pigmentation, as compared with the diploid larvae, but was otherwise apparently normal. The triplo-hexaploid condition probably resulted from chromosome duplication during the early cleavage of a triploid zygote.

SUMMARY

Whole-mount preparations of the tailtips of 312 larvae of *Triturus granulosus* Skilton were examined cytologically for the occurrence of spontaneous and cold-induced heteroploidy. On the basis of counts of chromosomes in a large number of metaphase plates of the tailtip epidermis, the diploid number of the species is confirmed as twenty-two.

One hundred twenty-eight of the larvae developed from eggs that had been refrigerated at various times after egg deposition. Of these larvae, 98 were diploid; 17 were triploid; one was tetraploid; 7 were haploid; two were haplo-diploid mosaics; and three were diplo-tetraploid mosaics. Of 184 larvae reared as controls for the experiment, there were 180 diploids, one pentaploid, two diplo-tetraploid, and one triplo-hexaploid.

Nuclear sizes in heteroploids of this species vary in relation to the number of chromosomes present; but by change in cell number, the body size remains near normal.

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THE SAVANNAH SPARROW (*PASSERCULUS SANDWICHENSIS* GMELIN) IN WINTER IN THE LOWER PIEDMONT OF NORTH CAROLINA

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INTRODUCTION.—The savannah sparrow (*Passerculus sandwichensis* Gmelin) is a common winter resident in suitable locations throughout North Carolina and the other southeastern states. In a previous study on the ecological succes-

sion of winter birds at Raleigh, N. C., in relation to the upland plant succession, the savannah sparrow was found to be the predominant bird in crabgrass (*Digitaria* spp., mainly *Digitaria sanguinalis*) fields, being abundant in crabgrass and present only in much reduced numbers in the other old field communities (Quay, 1947).

The purpose of the present study was to investigate in greater detail some of the more important factors influencing this narrow habitat selection by the savannah sparrow, especially habitat composition, population density, food habits, and behavior. A summary of the results was presented at the tenth annual meeting of the Association of Southeastern Biologists, Knoxville, Tennessee, April 15, 1949, an abstract of which was published in the Association's *Proceedings* (Quay, 1949).

METHODS.—All the fields used as study-plots were within a rectangular area of 15 square miles of agricultural land bordering Raleigh on the west. The physiographic location was in the extreme east-central edge of the lower Piedmont. About 70 per cent of the region was in cleared farmland, pastures, and abandoned fields; 30 per cent was in woody growth, mostly pines.

The field studies were conducted during the months of December, January, and February, in the winters of 1946–47 and 1947–48. Seventeen different fields, totaling 215 acres, were used as study-plots during the two winters. Eleven of these were in use in 1946–47 and 14 in 1947–48. Nine of them were under observation in both winters. Plot selection was necessarily influenced by availability. Plots containing some *Digitaria* were in the majority but there were one or more plots of each stage of the old field plant succession (Crafton and Wells, 1934; Oosting, 1942; Keever, 1950), plus some with mixtures of natural communities and agricultural plants (Table I).

Two hundred and eight field trips were made in all, of which 134 were specifically for population counts. A part of nearly every day each winter was spent in the field, regardless of weather conditions, conducting censuses, analyzing the

TABLE I

Summary of types, numbers, acreages, and censuses of field plots

Plot Type	Number	Acres in Plots	Censuses
Bare Field	3	30 (14, 11, 5)	10
Digitaria-Sorghum (mowed)	1	19	8
Digitaria-Medicago	4	46 (14, 14, 9, 9)	36
Digitaria-Lespedeza (low)	1	13	3
Digitaria and *Digitaria*-Tall Weeds	1	25	36
Tall Weeds-Rushes-Sedges-Grasses	1	12	7
Tall Weeds-Grasses (*Cynodon* and others, not primarily *Digitaria*)	3	32 (21, 7, 4)	9
Tall Weeds-*Andropogon*	1	13	7
Andropogon, with some Tall Weeds (one with some *Aristida*)	2	25 (22, 3)	18
9 Types	17	215	134

vegetation, observing behavior, or collecting specimens. On each census the plot was systematically examined by walking back and forth until completely covered, as in the earlier study (Quay, 1947). The distance between census lines and the time-per-acre spent varied according to visibility (height and density of vegetation), weather, time of day, etc. The use of this method with the savannah sparrow presupposed a rather detailed and special knowledge of the bird's habits.

Vegetational analysis was accomplished by use of typical phytosociological methods (Weaver and Clements, 1938; Oosting, 1956). The areal cover type of quadrat measurement was selected as most suitable (Bauer, 1943), 10 or more quadrats per plot, each quadrat one meter square (Oosting, 1942). Measurements were made at ground level, low grass level (which included *Digitaria*, *Cynodon*, *Setaria*, and *Eleusine*), and tall weed level. By such means it was possible to arrive at a quantitative measure of bare ground available and of overhead cover at various levels. The actual measurements were taken by direct visual estimate of the percentage of areal cover by species and levels (Penfound, 1945).

One hundred specimens were collected in each of the two winters. These were examined for sex, weight (1947–48 only), and food contents of the stomachs. The foods and feeding habits of the savannah sparrow will be discussed mainly in a future paper. Thirty-six of the birds shot between December 26, 1947, and February 18, 1948, were made into study skins; these skins were kindly identified to subspecies by Dr. John W. Aldrich.

SUBSPECIES.—Five subspecies of the savannah sparrow, in addition to the coastal *princeps*, occur regularly but in unequal ratios as winter residents and migrants in the southeastern states: *savanna*, *mediogriseus*, *nevadensis*, *labradorius*, and *oblitus* (Peters and Griscom, 1938; Aldrich, 1940; Wetmore, 1939, 1941; Pearson, Brimley, and Brimley, 1942; Burleigh, 1944; Johnston, 1956). An intensive analysis in South Carolina in 1956, involving 447 specimens live-trapped in January–February and identified by the new technique of field recognition of the living bird in the hand, demonstrated, ". . . complete spatial overlap of the races [all 5] on their wintering ground . . ." (Norris and Hight, 1957: 49).

The wintering population of the savannah sparrow at Raleigh proved to be a mixture of at least four subspecies. The 36 study skins were identified to subspecies as follows: *savanna*—11 males, 1 female, 1 sex undetermined; *mediogriseus*—6 males, 7 females; *labradorius*—8 males, 1 female; *oblitus*—1 male. If this small sample is adequate, and since it is characteristic of passerine birds in the United States for males to winter farther north than females (Nice, 1937), then it would appear that the central lower Piedmont of North Carolina is well within the wintering range of *mediogriseus*, in the northern part of the wintering ranges of *savanna* and *labradorius*, and on the periphery of the *oblitus* range. All three common races were taken in each of several different plots and also in the same plot at the same time. Since ecological analysis at the subspecific level was not attempted, the data and conclusions in this paper can necessarily apply only at the species level.

POPULATIONS IN HABITATS.—The average populations of the savannah

sparrow per acre in the various habitats for the two winters are listed and described below.

Bare Field.—None to a fraction of one per acre, according to the amounts of scattered tufts and clumps of unplowed *Digitaria*.

Digitaria.—Ten to fifteen per acre in well-developed, first-year fields; two to eight per acre in second-year fields, with increasing ground cover, decreasing *Digitaria*, and increasing tall weeds.

Digitaria-Tall Weeds, and *Digitaria-Medicago* (alfalfa).—Two to eight per acre. The wide range in these stages, as in the relatively pure *Digitaria* fields, was because the population varied in proportion to the amount and density of *Digitaria* present and to the density and arrangement of the total plant matrix, as will be more adequately described later.

Andropogon-Tall Weeds, and *Andropogon*.—None to one per acre, often one-tenth to seven-tenths per acre.

These population figures for 1946–48 agreed in detail with those obtained for savannah sparrows in the winter of 1939–40 in the same region (Quay, 1940). Because the earlier publication, which considered all the birds of the upland plant succession (Quay, 1947), did not list the savannah sparrow populations as such, they are given here for comparison: (1) Bare Fields—0; (2) Bare Fields, with scattered tufts of incompletely plowed *Digitaria* and sparse winter grain two inches high—0.50 per acre; (3) Dominantly *Digitaria* Fields, of all types and ages—8.4 per acre, up to 15 per acre in the most favorable plots, constituting four-fifths of all birds present; (4) Tall Weeds-*Andropogon*, and *Andropogon*-Scattered Low Pines—0.60 per acre.

Recent counts of savannah sparrows in old fields in the Savannah River Plant area of South Carolina (upper Coastal Plain, about 250 direct air miles southwest of Raleigh, N. C.) showed densities of 8 to 15 or more per acre in the more favorable parts of one large field and a general December-February average of 3.6–6.1 per acre (Norris and Hight, 1957: 49). These authors (*ibid.*: 50) suggest that their figures of 6.1 savannah sparrows per acre in December to early January and the grand average of 3.9 per acre for November to March may be close estimates of the wintering population in an abstract, heterogeneous community of old fields. At Raleigh, where the only old fields with more than one savannah sparrow per acre are those in which *Digitaria* is common to dominant, abstracting all the grassy and forby stages into a single community does not appear valid, either for savannah sparrows alone or for the total old field bird population (Quay, 1947: 387).

FOOD HABITS.—Previous studies on the food habits of savannah sparrows have been too few and scattered to be of much value in a selection versus availability study. There is no published information available on the foods or feeding habits of the savannah sparrow in the southeastern United States (Martin, Zim, and Nelson, 1951: 194–195).

The 200 savannah sparrows collected in the present study were selected numerically in approximate direct proportion to the types and acreages of the study plots and equally spaced throughout the two winter seasons. The speci-

mens were taken in the same plots as used for the population counts, so that the birds-per-acre figures for unshot fields might have been even higher than given. The 200 stomachs were analyzed by the aggregate percentage method (Martin, Gensch, and Brown, 1946).

The savannah sparrows ate about 97 per cent seeds and 3 per cent animal food. Thirty-six different species of seeds were taken in all, divided among 24 genera. *Digitaria* seeds formed 70 per cent by volume of all foods eaten, and appeared in all but two stomachs for a frequency of 99 per cent. Other genera with volumes more than 1 per cent in at least one winter were, in descending order of importance: *Sorghum*, *Ambrosia*, *Eleusine*, *Andropogon*, *Cyperus*, *Amaranthus*, *Setaria*, and *Aristida*. Savannah sparrows were very localized feeders. Under normal conditions, they fed on the ground and under the cover of the overhead foliage. Feeding was a continuous, all day process but the crop was filled only once a day, toward sunset.

SOCIAL BEHAVIOR.—The savannah sparrow proved to be at most only a weakly flocking species. Closely-knit flocks, of the type exhibited by field sparrows or starlings, were never observed. The arrangement was that of temporary, various-sized groups or loose aggregations. In the field notebook it was customary to write, for instance, "9 savannahs loosely aggregated in a space 60 feet in diameter." As come upon in the undisturbed state, savannah sparrows commonly were found from singly up to loose groups of 20 to 60. Most commonly the aggregation numbered fewer than ten birds. In contrast, all the field sparrows, or meadowlarks, in a particular field were in a single flock. The savannah sparrows, however, were spread more or less evenly throughout the field, a few here and a few there, much in the manner of insects or plants. In plots of low population they mostly occurred singly or in twos or threes. In plots of high population the groups were larger and more frequent but even there many individuals were definitely alone. The larger so-called flocks or groups apparently were usually composed of smaller groups and singles temporarily aggregated in the more favorable spots of food and cover. Groups of two or more were easily, too easily, broken up. Aggregations which it was desired to keep together for a while for study and census frequently dispersed into widely scattered smaller groups and singles.

There was one definite aggregating mechanism which served, though at times rather ineffectively, to maintain the weak type of flocking—the call note. The single call note was a faint and sibilant "tseep" (variations: tseeep, tseeh, tseeeh, tseh, tsip). The note was not given while the bird was on the ground and undisturbed. Typically, the first note was given just before or as the bird took flight and then an additional one each two or three seconds while in flight. This note had a disturbing or alerting influence on other savannah sparrows nearby. The note was usually effective as a signal to the others to follow the caller, not quickly and all at once but slowly and as singles and groups of two and three, which birds themselves also "tseeped" as they flew. This pattern is in accord with the behavioral theory of social releasers (Lorenz, 1937; Tinbergen, 1948).

It was evident also that some association was maintained on the ground by

sight, though vision was probably not very effective at ground level in the herbaceous vegetation except for relatively short distances.

When disturbed by a man walking, savannahs either moved onward on the ground or took flight. Flights were usually short, 20–70 feet, and practically never carried the bird out of the plot. Flight was quick, erratic, and only a few inches above the vegetation. During the periods of accelerated feeding and reduced light early in the morning and late in the afternoon, flushing distances were 12 to 25 feet. During the rest of the day the birds flushed on the average between 30 and 45 feet. The best time for censusing was between nine and ten o'clock in the morning.

Savannah sparrows occasionally occurred interspersed in the same local area with field sparrows, song sparrows, and juncos. These mixtures, on the part of the savannahs, were coincidental and not based on constant social interrelationships. Only a few dominance reactions (Odum, 1942) were observed. These were with juncos and song sparrows, and in every case the savannah was the vanquished. Dominance reactions were not ever seen among savannahs themselves. The opportunity for such contacts between savannah sparrows and field-edge, thicket, or woods-border birds rarely occurred, because savannahs invariably remained away from the very edges of the plots.

Meadowlarks occurred in all types of savannah sparrow plots, especially in the rather open *Digitaria-Medicago* ones. While groups of the two species did not mix, yet there appeared to be no limiting competition between them. Examination of twelve stomachs of meadowlarks from savannah sparrow plots revealed an entire lack of competition for food. Meadowlarks took larger seeds, cultivated grains, and more insects, and the only *Digitaria* seeds found were two in one stomach.

DAILY CYCLE OF ACTIVITY.—Under normal conditions savannah sparrows did not engage in local or long-distance movements during the winter. Populations remained uniform except under changing conditions of food, cover, and weather. Movements of groups from one plot to another were never seen. Indeed, it was practically impossible to chase a savannah sparrow out of its plot.

A special study of roosting habits was not made, but it is believed that the savannahs roosted on the ground in the same fields where they were in the daytime (Evans and Emlen, 1947: 5). They were found already in the plots and down on the ground as soon as visible in the morning. They were still in the plots and down on the ground feeding when last visible in the evening. These morning and evening points of change between visibility and invisibility coincided with the beginning and ending of civil twilight, at which time the sun is six degrees below the horizon and the measureable light is 0.2–0.6 foot-candles (Nice, 1943: 99–101). A number of light measurements were made with Weston photometers. It was quite light enough to see savannahs on the ground at 5–10 foot-candles. Shooting on the wing was possible at 2–5 foot-candles.

Savannah sparrows were engaged in accelerated feeding during the first two hours of light in the morning and the last two hours of light in the evening. During the rest of the day the birds continued to remain out of sight on the ground,

fed in regular but more leisurely fashion, were in larger and stronger aggregations, flushed more quickly and farther away, and did more "tseeping." Occasional individuals did perch on top of the vegetation in late morning, as if sunning. Bathing was seen only once, in a puddle.

The savannah sparrow was not an easy bird to watch. When disturbed, it ran on the ground more often than it flushed. Crouched low to the ground, head down and stretched forward, it ran quickly and quietly, taking advantage of all cover and resembling a mouse more than a bird. Its habits in this regard were found to be in close agreement with those described in the general literature.

RESPONSE TO ABNORMAL WEATHER.—In 1946–47, December and January were unseasonably warm and there was an abundance of savannah sparrows in all favorable plots. While February, on the other hand, was colder and drier than normal the savannah sparrow population did not noticeably decrease. There was only a trace of snow all winter. The single ice glaze, on February 20, lasted 36 hours. It was confined to the vegetation above ground, the ground itself remaining practically free.

The winter of 1947–48 was on time and approximately normal in temperature. From the start, the savannah sparrow population was only one-half to two-thirds of that of 1946–47. This may have been due to the earlier and colder winter temperatures resulting in more birds wintering farther south. These yearly differences account partly for the rather wide ranges in number of birds per acre listed in the population section.

The month from January 14 to February 14, 1948, was a time of continuously below-normal temperatures and repeated sleetfalls, snowfalls, and ice glazes. This prolonged seige of cold, snow, and ice covered the entire eastern and central United States and was composed of a series of cold waves emanating from the far northwestern part of the continent. During January 14–23 there were daily freezing temperatures, frequently down to 10–15 degrees Fahrenheit. This sharp drop in temperature alone caused no visible change in the savannah sparrow population. On the 24th of January two inches of snow and fine sleet fell, accompanied by a glaze of ice on the vegetation. The sleet and snow covered the bare ground completely through the 26th, and thirty per cent of the ground still by the 30th. The ice glaze melted by the afternoon of the 25th.

The savannah sparrows were entirely gone on the 25th from all the Tall Weeds and *Andropogon* Plots, from all the *Digitaria-Medicago* Plots except for 24 birds in a sheltered spot of *Digitaria* in the lee of a Tall Weeds' edge in Plot 11, and from the mowed *Digitaria-Sorghum* Plot. Thereafter until the middle of February, except for 2 birds in Plot 11 on February 6, none of these plots contained any savannah sparrows. During the clear and warmer weather of the second half of February there was only about 25 per cent recovery. The alteration of habitat was especially clear in the *Digitaria-Medicago* Plots, where the substratum was changed from a protectively-colored dark brown to a glistening white and the already low canopy of 3–6 inches was further reduced to 1–3 inches. All vegetation in these plots was entirely covered by an 8-inch snow on February 1 and again on February 9–10.

Plot 9 was a 25-acre field of well-developed *Digitaria* and *Digitaria*-Tall Weeds mixtures, constituting the largest single unit of optimum savannah sparrow habitat under study. Total populations counted in this field in 1946–47 ranged from 225 to 390, usually about 300–350. Similarly, in 1947–48 through January 19 the counts ranged from 175 to 250, being less both because of habitat deterioration for savannahs and because of the general regional population decline. In this plot there was an initial increase in population on January 25, 1948, to half again as much as earlier in the season, dwindling to one-half as much by the 31st. Under the impact of more ice glaze, sleet, and the eight inches of snow on January 31–February 1, the savannah sparrows were reduced to about one-tenth their earlier numbers. Recovery here also was practically non-existent during the second half of February. The few birds present during the first two weeks of February existed under what was for them greatly modified conditions. For days, almost their only food was *Ambrosia* seeds picked from the lowest stems by stretching upwards while standing on the ground or fluttering up a few inches off the ground. *Sorghum* and *Amaranthus* seeds were also taken from the plants, when these were free from ice. There was a nearly complete return to *Digitaria* as soon as isolated spots became exposed in the melting snow.

The savannah sparrows which disappeared could have either died or moved farther south. Careful search was made for dead birds, both in the regular and other habitats, but none were found. The writer believes that the savannahs made a wholesale, mid-winter, southward "weather movement" (Thomson, 1926: 115–116). Such behavior apparently is not atypical for savannah sparrows in the eastern United States. Cruickshank (1942: 451) for Long Island reported: "After the first killing frost in early November numbers quickly fall off, and by the end of the month the species is casual in the interior. Along the coast, however, flocks regularly linger until Christmas, and in mild seasons small groups remain throughout the winter." Witmer Stone (1937: 900) found the same situation at Cape May, New Jersey: "Savannah Sparrows are most plentiful in the spring and autumn migrations but a few remain through every winter and in some seasons they occur in large numbers, as in the week of January 29, 1891, when Samuel Rhoads and I found them fairly swarming on the meadows between Cape May and the Point during a spell of extremely cold weather with a gale from the northwest."

NICHE RELATIONSHIPS.—The evidence from the present study, as given in the foregoing sections, showed that savannah sparrows in the Raleigh region were limited almost exclusively to fields of *Digitaria*, within which habitat they remained primarily down on the ground and out of sight under the canopies formed by the loosely intertwined stems of grass. Within this one habitat savannahs apparently experienced adequate fulfillment of all their major winter-time needs: food, suitable feeding conditions, continuous protection from enemies, protection from the elements, and roosting sites. Savannahs were present in *Digitaria* fields at all times, whether early or late, warm or cold, wet or dry, windy or calm, and whether disturbed or undisturbed. No other bird of the old field plant communities was characterized by such a consistently narrow and rigid habitat selec-

tion. No other bird was found continuously widespread and abundant throughout all parts of large fields of *Digitaria*. The other sparrows (field, song, white-throated, junco) were confined largely to the field edges and invariably flew to thickets and woods-borders upon being disturbed. Meadowlarks chose more open habitats, as bare fields, winter grain fields, and the rather sparsely vegetated *Digitaria-Medicago* fields.

By means of the areal-cover, meter-square quadrats and additional visual estimates in local areas, it was possible to derive a quantitative measure of the structure and composition of the most favorable savannah sparrow habitat, i.e., of that environment in which the highest numbers of savannahs consistently occurred, as follows: A *Digitaria* consocies, mediumly dense and 15–20 inches high. Plants of typical form, the main stems few below but branching and spreading above, resulting in a canopy of slender stems and digitate racemes at 8–15 inches off the ground. Ample room under this canopy for savannahs to move about freely, the main stems, other plants, and litter accounting for only 10–40 per cent of the ground area, leaving 60–90 per cent of the ground surface bare and available. Overhead areal cover (canopy) of *Digitaria* about 80 per cent (70–100 per cent), dense enough to be an effective overhead screen but loose enough to admit considerable light and to permit quick entrance and exit by the sparrows. The overhead areal cover occasionally as low as 20–40 per cent, if arranged in an interlocking fashion, like city streets, providing some cover over or near birds at all times. The thickest growths of *Digitaria* not the most favorable, because of their lowered canopies and decreased room for free movement (stems too close together). Scattered tall weeds when present lending physical support to *Digitaria* clumps, especially in late winter, but not necessary. Dense growths of tall weeds and other forbs and grasses reducing and eliminating *Digitaria*, resulting in increased ground litter. *Ambrosia* the only tall weed the seeds of which are regularly used as food, and *Sorghum* the only tall grass of similar use.

Thus, the habitat niche of the savannah sparrow in winter around Raleigh was found in the ground-level stratum of a particular facies (*Digitaria*) of one life form of vegetation (grass). This niche was composed in large measure of: (1) bare ground on which to move and forage, (2) an abundance of small seeds easily visible on the ground surface and available without scratching, (3) an overhead cover of low to mediumly tall grass.

SUMMARY

Population density, habitat composition, food habits, and behavior were investigated as prime factors influencing the narrow habitat selection exhibited by savannah sparrows in winter. The field research was conducted during the months of December, January, and February in the winters of 1946–47 and 1947–48. Average populations in habitats were: 10–15 per acre in well-developed, first-year *Digitaria*; 2–8 per acre in mixed *Digitaria*-Tall Weeds, and in *Digitaria-Medicago*; 0–1 per acre in mixed Tall Weeds-*Andropogon*, in *Andropogon*, and in relatively bare fields. The most important food item throughout the winter

was *Digitaria* seeds (70 per cent of total volume). The single "tseep" note served as a partially effective aggregating mechanism and helped to maintain a loose type of flocking behavior. A three-weeks' period of nearly continuous snow, sleet, and ice glaze resulted in reduced populations and a temporary change in feeding habits. Savannah sparrows stayed primarily on the ground and under cover at all times. Mediumly dense stands of *Digitaria* constituted the most favorable winter habitat of the savannah sparrow in the area of study, supplying the essential niche requirements for this bird of small seeds, bare ground, and overhead cover. The physical structure of the *Digitaria* plant community is described, especially in relation to savannah sparrow behavior.

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NORTH AMERICAN LEJEUNEACEAE

VI. LEJEUNEA: SUBGENUS LEJEUNEA (II; CONCLUDED)*

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Lejeunea glaucescens Gottsche

Lejeunea glaucescens Gottsche, *in* G. L. et N., Syn. Hep. 378, 1845; Evans, Bull. Torrey Bot. Club. **32**: 188, 1905; Evans, Amer. Jour. Bot. **5**: 136, 1918; Frye and Clark, Univ. Wash. Publ. Biol. **6**(5): 802, figs. 1–12, 1947 (in part?).[32]

Lejeunea (*Eu-Lejeunea*) *glaucescens* Stephani, Hedwigia **29**: 85, 1890.

Plants in flat, loosely adnate patches or mats, *pale or whitish green, opaque, dull or only slightly nitid*, rather delicate and lax, but *relatively large: main shoots usually 1.0–1.4 to 1.4–1.6 mm. wide*, to 15–18 mm. long, irregularly and sparsely branched (but often *with numerous sexual branches*); stems 90–110 μ in diam., the *ventral cortical cells averaging 32–36 μ wide*. Leaves approximate to slightly imbricate, obliquely to widely spreading (50–70°), little convex, nearly flat in drying, but with margins not becoming reflexed, *the margins weakly crenulate*; dorsal lobes *widest virtually at base, ovate to ovate-triangular, occasionally dimidiate-ovate*, up to 540–650 (800) μ long × 500–540 (640) μ wide (*length 1.05–1.3 × width*); anterior margin strongly arched upward and outward at the very base, then evenly curved to the *narrowly rounded to obtusely pointed apex, the leaf widest virtually at base*; posterior margin varying from nearly straight to slightly convexly arched. *Lobules polymorphic:*

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* Continued from **73**(1): 122–197.

[32] The writer is uncertain whether the figures and description in Frye and Clark pertain to this species. The cells of the perianth-mouth are never as sharply differentiated as suggested in their Fig. 7, nor are the female bracts ever as nearly equally bilobed as in their Fig. 4. They state that the underleaves are 85–120 μ long × 75–105 μ wide, and use the small underleaf size as one of the "key characters" in separating this species from *L. cladogyna*. In point of fact, *L. glaucescens* has on an average much larger underleaves than *L. cladogyna*. As a consequence, *L. glaucescens* of Frye and Clark is a dubious entity.

occasional ones (rarely most!) small and reduced to a basal flap, but almost *uniformly inflated on most plants*; well-developed lobules *small* (*length ca. ¼ length of dorsal lobe*), 130–150 (180) μ long × 100–130 μ wide at the base, *very broadly triangular-ovate*, strongly inflated along keel only, the line of insertion on the stem long (subequal to keel usually); free margin involute only near base, apical region more or less flatly appressed to lobe, the apical sinus very oblique, very shallow; *keel varying from straight to barely convex*, the posterior leaf-margin hardly notched at juncture of keel and dorsal lobe. Cells thin-walled, with small to minute trigones and rare to occasional intermediate thickenings; *marginal cells averaging 20–25 μ* (on mature leaves), *the median 24–28 × 33–38 μ*; *oil-bodies 4–10* per cell, rarely more, distinctly *segmented*, 3 × 5–8 μ up to 4–5 × 7–10 μ. Underleaves *distant* (separated by intervening distance usually at least equal to the underleaf-length), medium-sized, *suborbicular to ovate*, 1.5–2.4 times as wide as stem, on mature stems usually 200–225 (250) μ wide × 230–250 (290) μ long (*length [1.0] 1.1–1.2 times the width*); underleaves averaging 2–4 × the area of well-developed lobules; sinus narrow, narrowly V- or U-shaped, descending through 0.5–0.6 of the underleaf-length, the erect to suberect lobes entire-margined to faintly crenulate, *5–6, occasionally 7(–8) cells wide at base*; rhizoid-initial disks usually small, of up to 16–20 cells.

Autoecious. Androecia uniformly on *short, leafless lateral* branches, 320–360 μ wide, usually of 3–4 pairs of bracts, but occasionally longly spicate and of 6–15 pairs of bracts, not or very rarely proliferating at apex; bracts strongly concave, subequally bilobed, with rounded lobes, 1–2-androus. Female inflorescences on a *very short lateral branch*, usually lacking normal vegetative leaves (very exceptionally an occasional one on an elongate, leafy shoot); subfloral innovation single. Bracts exceedingly variable, *usually much smaller than mature vegetative leaves*, often the two bracts of a single inflorescence greatly differing in size (the smaller with a lobe *ca.* 320–340 μ long × 120–180 μ wide, lobule 200–220 μ long × 40–50 μ wide; large bract with lobe 520–540 μ long × 190–220 μ wide, the lobule 170–280 μ long × 50–80 μ wide); lobe of bract narrowly obovate to lingulate, obtuse to rounded (rarely acute) at apex; *lobule very variable, usually small, free for 0.25–0.6 of its length, sublinear*, acute to narrowly rounded at apex. Occasional mature gynoecia with one or both subtending bracts even more reduced: the lobe merely 175 μ long × 110 μ wide, the lobule then subequal in length, 150 μ long × 75 μ wide. Bracteole (on smaller inflorescences) only 280 μ long × 170 μ wide, oblong, up to 350 μ long × 240 μ wide, *bifid ca. ⅓, the lobes erect or connivent, acute*. Perianth long-exserted at maturity, 0.5–0.75 emergent, *obovate-clavate*, not or weakly compressed, to 400–430 μ in width × 700–800 μ long, the *distal one-third 5-carinate* (when mature; when immature keeled to the middle), the antical keel often low, the lateral keels (and to a less degree the ventral) sharp, compressed, crenulate with bulging cells, *not dilated into horns*, but the short beak often somewhat recessed at the *truncate apex*.

TYPE: Brazil.

DISTRIBUTION.—A polymorphous, widespread tropical species, evidently of considerable antiquity. Found throughout much of tropical South America (from Brazil northward), in the West Indies (Puerto Rico, *fide* Pagan; St. Thomas, Virgin Isls., Evans, 1918; Great Bahama and Cat I., Bahama Isls., *fide* Evans, 1911; Bermuda, *fide* Evans, 1906), and to a restricted extent in the southeastern United States, where it is found in both the tropical and subtropical portions of the Coastal Plain. The species proper is confined, in our region, to Florida and southern Louisiana, but the var. *acrogyna* is a disjunct on the inner coastal plain of North Carolina.

Florida: DADE CO. (Deep hammock forest, east side Long Pine Key, 1 mi. n. of Firetower, Everglades Ntl. Park, *RMS 36812, p.p.*, among *Rectolejeunea phyllobola* and *RMS 36822a, p.p.*, among *Cololejeunea subcristata*; Timms Hammock n. of Bauer Drive, n. of Homestead, *Small, Mosier & Small 6180!* [plate]; Paradise Key, Everglades Ntl. Park, *RMS 19928*,

22603; Mahogany-Royal Palm Hammock just s.w. of w. tip of Long Pine Key, w. edge of Dade Co., in Everglades Ntl. Park, *RMS 37345, 37338, 37365c*; Brickell Hammock, s. edge of Miami, Biscayne Bay, *Britton 32!*; on twigs, hammock on Loveland Rd., *ca.* 5 mi. w. of Florida City, *RMS 22700, optimal!*; Mathesson Hammock, near Coral Gables, *RMS 33899a, 22632, p.p.*, among *Lejeunea minutiloba*; Ross and Costello Hammocks, near Hainlin Drive, *RMS 22782, etc.*; Hammock in Section 24, Long Key Pineland, Everglades Ntl. Park, *RMS 22023*); COLLIER CO. (Collier-Seminole State Park, near Royal Palm, *RMS 27861, etc.*; Deer Lake, Big Cypress Swamp, *McFarlin & Grout 1035, fide* Frye & Clark, l.c.); GADSDEN CO. (Pronto Springs, Kurz & Little, 1933); HILLSBOROUGH CO. (Hillsborough R. State Park, *RMS 33921c, 33921b, p.p.*, with *L. cladogyna*); ALACHUA CO. (Devils Millhopper near Gainesville, Kurz & Little, 1933); SEMINOLE CO. (Sanford, *Rapp 55!* [plate]). Also reported from Highlands and Polk Cos. (Redfearn, 1952). **Louisiana:** Avoca and Bateman's Island (Svihla, 1939). The species was also reported from Rutherford Co., Tennessee by Morrison (1938) and Sharp (1939).

There is only a single voucher specimen examined by Redfearn in the University of Tennessee Herbarium. This is from Polk Co.; it is very clearly *Rectolejeunea brittoniae* Evs., as is obvious from these features: leaves freely caducous; dioecious; leaves often with lobules vestigial near caducous regions, and then lobes with posterior margin spreading at an angle of 95–110° near postical base. The Redfearn (1952) reports from Highlands and Polk Cos. are therefore not authentic.

Dr. A. J. Sharp kindly loaned to the writer the single collection from Tennessee, since this report of the species is totally away from the Coastal Plain, and therefore was regarded as questionable. Examination of the material revealed the following characteristics: (a) underleaves large, broader than long, with an arched and decurrent line of insertion, with broad lobes (up to 8–9 cells broad at base) and a lunate sinus; (b) cells with obvious trigones and very frequent intermediate thickenings; (c) perianths on short lateral branches, with virtually no exceptions, with single innovations that were usually again fertile, sometimes repeatedly so; (d) very large, pellucid cortical cells, those of the ventral face 34–45 μ broad, up to twice the diameter of the underleaf cells. These characteristics, particularly those of the underleaves and cells, suggest strongly that a species of *Taxilejeunea* is at hand, certainly not *L. glaucescens*. This problem will again be discussed in conjunction with the future treatment of *Taxilejeunea*.

ECOLOGY.—One of our more mesophytic species of *Lejeunea*, occurring perhaps most frequently (in our area) on damp, corticated or decorticated, decaying logs, lying in dense evergreen forests. On fallen twigs, branches or logs associated with a wide variety of other bryophytes, among them (as in *RMS 33921b: Aphanolejeunea evansii, Lejeunea cladogyna, Ceratolejeunea laete-fusca, Cheilolejeunea rigidula, Cololejeunea setiloba* and *Fissidens radicans*, as well as (*RMS 37338*) *Cololejeunea subcristata* and *diaphana* or (*RMS 36812a* and *36822a*) *Cololejeunea subcristata, Rectolejeunea phylloloba* and *Caudalejeunea lehmanniana*. The species, however, often forms very large and extensive, somewhat pale to light yellow-green, dull, pure patches on decaying logs. Indeed, in southern Florida it often is the dominant species to undergo ecesis on shaded logs or fallen tree-trunks, particularly in the deep, tropical "hammock" forests.

Also occurring on calcareous rocks (often coral limestone), and on the bark of

trees, where it is found both on the trunks of trees, and on branches or twigs, but more commonly on the deeply shaded hammock floor (on dead twigs and branches), where the humidity is exceptionally high. There sometimes accompanied by other species of *Lejeunea* (*L. minutiloba*, as in *RMS 22632*). The species also occurs occasionally on the exposed bark of trees (Royal Palm, in *RMS 19928*); there it may be associated with other *Lejeuneae*, among them *L. flava*, *Taxilejeunea sp.*, *Cheilolejeunea cf. polyantha*, *Lejeunea bullata*. When occurring under relatively exposed conditions, the plants are almost uniformly lobulate (even on fertile plants, which otherwise often bear, in part, virtually elobulate leaves).

DIFFERENTIATION.—*L. glaucescens* is most aptly characterized by the following combination of features: segmented oil-bodies (Fig. XXXVI: 11); dull texture when dry; distant, relatively small underleaves (Fig. XXXV: 1, 9–10); a rather robust size (mostly 1–1.35 mm. wide on mature shoots, but exceptionally up to 1600 μ wide on robust shoots); ovate to almost dimidiate-ovate leaves which tend to be slightly convex when dry, average clearly longer than broad, and are perceptibly narrowed from just above the dilated antical base on (Fig. XXXV: 8–10; XXXVI: 1, 5); a relatively high incidence of inflated lobules, which are, however, small in size (up to $\frac{1}{4}$ the length of the dorsal lobes); constantly or almost constantly cladogynous female inflorescences, with the bracts usually greatly reduced in size (Fig. XXXV: 1). The high incidence of inflated lobules is quite distinctive. This might lead to confusion (in the southeast) with *L. flava*. The latter species, however, has much larger, subcontiguous to imbricate underleaves, is more pellucid and shiny, and has larger lobules (in general). *L. glaucescens* is apparently most closely related to *L. calcicola*, with which it also agrees in the general size and form of the underleaves. However, the broader-than-long, basally rounded to subcordate underleaves of *L. calcicola* are distinct from the orbicular or ovate, often slightly longer-than-broad underleaves of *L. glaucescens* (Fig. XXXV: 9–12; XXXVI: 2–3).

Lejeunea glaucescens also appears to be distantly allied to *L. floridana* (as has been stressed by Evans, 1905, p. 188). It differs from the latter species in the smaller cortical cells of the stem (averaging under 36 μ wide on mature stems, ventrally); in the ovate to ovate-orbicular, rather than transverse underleaves; in the cladogynous inflorescences with small bracts (and long-exserted perianths); in the retention of distinct lobules of the female bracts, which are often sharply pointed and free for at least a third of their length (Fig. XXXV: 4–7); and in the form of the perianth. The latter, in *L. glaucescens*, is truncate at the apex (because of the slightly dilated and abruptly terminated keels), and may have the short beak slightly recessed (Fig. XXXV: 1); in *L. floridana* the keels are slightly produced as rounded shoulders, usually to a point exceeding the apex of the beak. Evans (1905) also states that the androecia of *L. glaucescens* are shorter and broader than those of *L. floridana*. However, in typical *L. glaucescens* (determined as such by Evans), I have found slenderly spicate androecia with as many as 15 pairs of bracts, and in typically developed plants (*RMS 36812*) there may be 12–14 pairs. This difference, therefore, is at best an inconstant one.

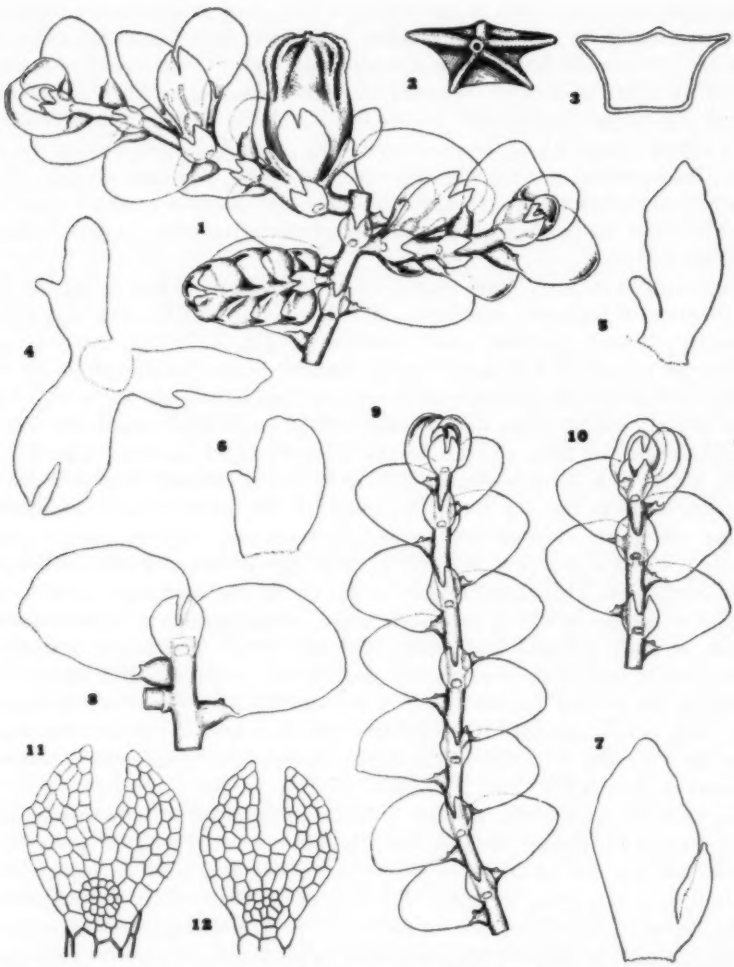


FIG. XXXV. *Lejeunea glaucescens* G. 1. Part of robust fertile shoot, with *Taxilejeunea*-like orientation of gynoecia (×30); 2. Apex of perianth (×48); 3. Cross-section of perianth, 0.4 from apex (×48); 4–7. Bracts; in 4 also with a bracteole (×60); 8. Postical aspect of shoot-sector, showing base of branch (×38); 9–10. Terminal portions of large, sterile shoots, postical aspect (×22); 11–12. Typical mature underleaves (×105). (Fig. 1, Sanford, Florida, *Rapp, 1912*; 2–12, Timms Hammock, Florida, *Small, Mosier and Small 6180*).

In the nearly constantly cladogynous female inflorescences, and in the highly reduced female bracts (correlated with which the perianth is quite exserted), *L. glaucescens* closely approaches the smaller *L. cladogyna*. However, the ovate to dimidiate-ovate leaves, narrowly rounded apically or even obtusely pointed; the

much more robust size; the greater frequency of inflated lobules; the larger cell size (of both marginal and median cells); and the relatively small lobules of the majority of female bracts all suggest that the two species are not at all closely allied. The dry plants of *L. glaucescens* (and the allied *L. calcicola*) are dull or scarcely glistening; those of *L. cladogyna* (and the very similar *L. minutiloba*) are, by contrast, extremely nitid.

As in most of our species of *Lejeunea*, the female bracts and the position of the female inflorescences are subject to considerable variation in *L. glaucescens*. The species at least occasionally produces a sporadic inflorescence on an elongate, leafy stem (but never regularly produces such inflorescences, unlike *L. calcicola*).[33] In general, the leading stem is "pinnulate" with short lateral branches lacking normal leaves, which produce either gynoecia or androecia. The gynoecial branches innovate, with the innovations often elongating, and at times again developing gynoecia. In the latter case, which is admittedly sporadic in occurrence, a *Taxilejeunea*-like orientation of gynoecia is sometimes approached (Fig. XXXV: 1). However, there is one critical distinction: in *L. glaucescens* the lateral, gynoecial branches produce generally fertile innovations, but these innovations are pinnulate often on both sides with short and virtually sessile gynoecia and androecia, while in *Taxilejeunea* the gynoecia are strictly on one side of the stem. In many cases the leading stem remains sterile and may grow indefinitely; in at least occasional cases it produces an acrogynous gynoecium, and then innovates. *L. glaucescens* also shows a bewildering degree of variation in size and form of the female bracts and bracteoles. This is most readily evident from Fig. XXXV: 4–7. Several generalizations appear to be valid, however: (a) the bracts and bracteole are essentially free from each other; (b) the bracts are never dentate or repand-dentate, they are usually obtuse to rounded at the apices, although occasional ones are bluntly acute; (c) the lobules vary from lingulate to narrowly lanceolate; they are most often acute or subacute, but may be narrowly rounded at the apex; (d) the lobules are small, generally less than 0.55 of the length of the lobes, except on bracts which are exceedingly reduced in size; (e) the perianths are long-exserted, usually for ½–¾ of their length, in spite of variation in bract size.

Lejeunea glaucescens also appears very variable with respect to lobule development. The common manifestations of the species usually show either uniformly lobulate leaves (Figs. XXXV: 1, 9–10; XXXVI: 1, 5), with the lobules characteristically short, yet broad (and with a broad line of attachment to the stem), or have only sporadically reduced lobules. However, certain extreme forms of *L. glaucescens* may have wholly or almost wholly "elobulate" leaves, possibly associ-

[33] A few such inflorescences, terminal on long leafy shoots, have been seen in *Schuster 19928* (Paradise Key, Fla.). These plants grade slightly into *L. calcicola* in the underleaves, which may be barely wider than long (up to 180 μ wide × 170 μ long, occasionally only 150 μ wide × 136 μ long) but are more often rotund (*ca.* 150 μ wide and long); they have unusually distinctly crenulate margins and an unusually broad, often almost lunulate sinus. They are further atypical in the relatively small cells (often averaging barely 21–23 × 32–34 μ). The plants, otherwise, have the aspect and size of *L. glaucescens*, and a similarly high incidence of lobulate leaves (reduced-lobulate leaves being exceptional, largely restricted to fertile plants).

ated with growth under extremely shaded or moist conditions. The "lobulate" extreme is apt to be mistaken, at times, for the otherwise very dissimilar *L. flava*; the "elobulate" phases for *L. setiloba* or even the otherwise quite different *L. minutiloba* and *L. cladogyna*. Unlike *L. setiloba*, the vestigial lobules have apical teeth formed of only 1–2 cells. Unlike the last two species, the lobules are not strictly dimorphic, but show a wide range of variation from large to extremely minute.

The plants from Sanford, Florida (*Rapp, January 1912*, NYBG) are of some interest in possessing several deviant features, among them: (a) large underleaves that are in some cases 290 μ long × wide; in other cases they are divided through 0.7 of their length with narrowly triangular lobes; in the wider underleaves the lobes are 7–8 cells broad; (b) female inflorescences, although overwhelmingly cladogynous, occasionally on long, leafy shoots; (c) cladogynous female inflorescences with their innovations usually floriferous; (d) inflorescences of innovations with often larger than normal bracts, the lobe of the bract up to 650 μ long × 330 μ wide, the lobule 270 μ long × 90–100 μ wide; the bracteole 550 μ long × 250 μ wide. In spite of these differences, the cell-size, leaf- and lobule-form, form of the bracts and perianth are typical, with the consequence that no other disposition of the material is possible. (It was also considered to represent *L. glaucescens* by A. W. Evans.)

Slightly deviant, also, are the plants from Hillsborough R., Florida (*RMS 33921b*). These are, in part, from a rather dry log, and as a consequence show cells with rather discrete trigones and frequent or occasional intermediate thickenings. The plants also very rarely possess a gynoecium situated on an elongate, leafy branch. Indeed, such acrogynous gynoecia occur rarely to sporadically in most of the more copious collections (*Rapp, 1912; RMS 33921b; RMS 37345*, etc.) which have been studied in detail, in spite of the fact that the female branches have been considered to be "constantly" abbreviated (e.g., by Evans, 1918, p. 136, and Frye and Clark, 1947, p. 803).

The writer has studied a long series of specimens of this common and polymorphous tropical species, and finds that it is distressingly plastic in many of its features. As a consequence, recognition of its more extreme phases will remain difficult, in spite of the distinctive facies of the species. Two extreme phases, difficult to interpret as mere habitat modifications, are accepted here as discrete varieties. These plants are more readily confused with other *Lejeunea* species than with *L. glaucescens*, s. str. These variants are treated as follows:

KEY TO VARIETIES OF *L. GLAUCESCENS*

1. Plants in large part with leaves lobulate, the lobules largely or wholly inflated; reduced lobules, if present, with apical tooth usually 1-, rarely 2-celled; generally more robust: 1–1.6 mm. wide; ventral cortical cells usually 30–36 μ wide; underleaves with lobes usually (5) 6–8 cells broad at the base ..... 2.
2. Almost uniformly cladogynous; bracts short, smaller than leaves, the mature perianth at least half emergent; androecia always on short lateral, leafless branches; ♀ bracts with lobules usually quite distinct ..... *L. glaucescens*, typical
2. Freely with acrogynous inflorescences (some of which with 2 subfloral innovations); such inflorescences with bracts large, as long as or longer than leaves, the mature

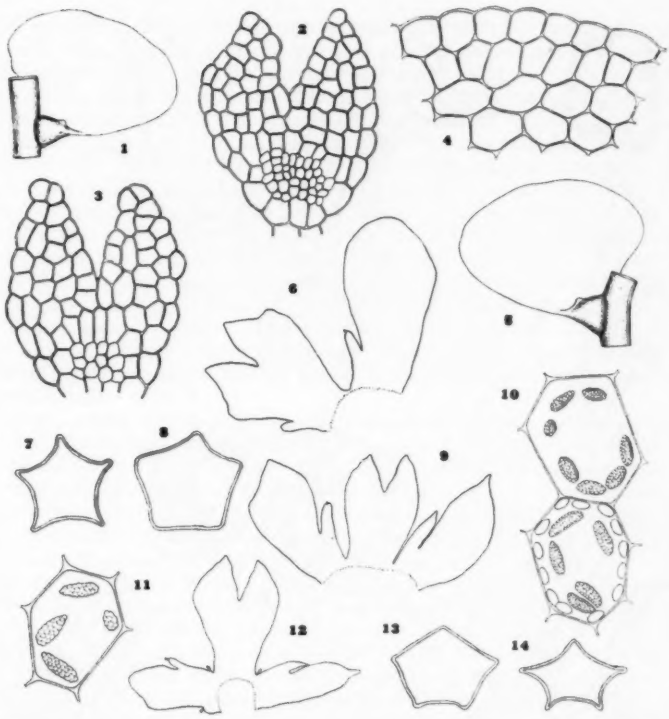


FIG. XXXVI. *Lejeunea glaucescens* G. 1, 5. Typical leaves (×30); 2-3. Underleaves (×112); 4. Marginal cells of leaf apex (×285); 6. Large bract and bracteole, from an inflorescence with perianth less than one-half emergent (×60); 7-8. Cross-sections through upper one-third and middle, respectively, of perianth (×43); 9. Reduced bracts and bracteole from a normal, cladogynous inflorescence with longly emergent perianth (×52); 10-11. Median cells with oil-bodies (10, ×535; 11, ×650); 12. Reduced bracts and bracteole from cladogynous inflorescence (×51); 13-14. Cross-sections through, respectively, middle and upper one-third of perianth (×40). (Figs. 1-5, Sanford, Florida, *Rapp, 1912*; 6-8, 12, Paradise Key, Everglades, Florida, *Schuster 19928*; 9, 11, 13-14, Paradise Key, *Schuster 33899a*; 10, Mathesson Hammock, Florida, *Schuster 33899a*).

perianth not emergent or hardly so; androecia variable in position, some terminal on long, leafy shoots; ♀ bracts with lobules usually reduced to a filiform appendix. *L. glaucescens* var. *acrogyna*

1. Plants with uniformly vestigial lobules, their apices bearing an erect tooth formed of 1-2 superimposed cells; smaller, 920-1100 μ wide usually; ventral cortical cells 23-25 μ wide; underleaves with lobes 4, rarely 5, cells broad. Gynoecia and androecia indiscriminately terminal on long, leafy shoots, or forming short, lateral shoots, as in var. *acrogyna* ..... *L. glaucescens* var. *obsoleta*

Lejeunea glaucescens var. **obsoleta** var. n.

Formae typicae similis sed differt androeciis et gynoeciis saepe terminalibus in caulibus ducentibus; lobulis obsoletis, dente apicali saepe 2-cellularibus;

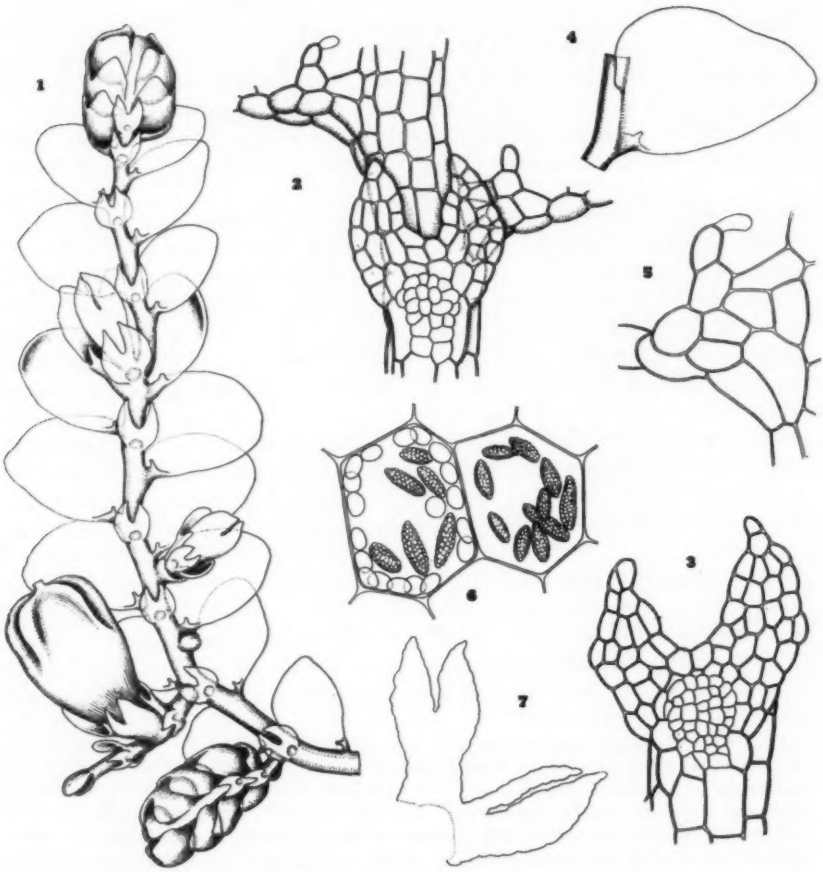


FIG. XXXVII. *Lejeunea glaucescens* var. *obsoleta* Schust. 1. Shoot, with lateral and terminal androecia and gynoecia (×42); 2. Stem sector with underleaf and typical lobules (×195); 3. Underleaf (×180); 4. Mature leaf, antical aspect (×44); 5. Typical lobule (×290); 6. Two median cells with oil-bodies, and, at left, with chloroplasts (×780); 7. Bract and bracteole (×69). (Figures all drawn from type, *Schuster 36860*).

amphigastria ovatis, sensim longioribus quam latioribus, lobis 4 (–5) cellulae latioribus; cellulae superae et marginalis 17–20 (23) μ. Specimen typicum legit R. M. Schuster, no. 33860, "Mathesson Hammock" prope "Coral Gables, Dade Co., Florida."

Plants in prostrate patches, almost pure green to slightly yellowish-green, *dull when dry*. Shoots when mature mostly 920–1100 (1250) μ wide, rather sparsely branched (except for sexual branches and innovations). Stems 64–75 (95) μ in diam.; ventral cortical cells mostly 23–25 (30) × 32–48 μ long. Leaves approximate to subimbricate, the lobes *ovate*, widest slightly above base, *narrowly rounded to obtuse at apex*, 360–380 μ wide × 480–520 μ long, occasionally

up to 425 × 550 or 500 × 720 μ; *lobules uniformly vestigial*, reduced to minute folds of 6–10 cells, bearing at the apex an *erect tooth formed of 1–2 superimposed cells*, terminated by the hyaline papilla, the tooth close to and more or less parallel with the stem. Cells moderate in size, *densely chlorophyllose, exceedingly leptodermous*, without distinct trigones or intermediate thickenings; marginal cells 17–20 (23) μ near leaf-tip, slightly bulging (the leaf-margins weakly crenulate); median cells (18–20) 21–24 × 24–29 μ; *oil-bodies segmented*, in most median cells narrowly ovoid or ellipsoid to fusiform, 3–3.5 × 7–10 μ, a few up to 4 × 12 μ, *mostly 5–12 per cell* (in frequent cells 12–18 or to 24 smaller oil-bodies, these only 2.5–4 μ and spherical to 2.5–3.5 × 3.5–5 μ and ovoid); chloroplasts 3.5–4 μ long. Underleaves varying from subequally long and wide (120–140 μ long and wide) *to somewhat longer than wide* (115 μ wide × 125 μ long to 125–130 μ wide × 150–160 μ long), *usually 1.3–1.8 times width of stem*, divided for 0.55–0.7 of their length into two erect, narrow, lanceolate-triangular lobes, *4, rarely 5 cells wide at base;* lateral underleaf margins evenly arched or *occasionally angulate-dentate; lobes frequently to usually tipped by 2 superimposed cells; sinus usually U-shaped to lunate.*

Autoecious, freely fertile. Androecia compactly spicate, either on short, leafless lateral branches (then usually of 2–4 pairs of bracts), or *on leading leafy branches* (then often longly spicate, up to 6–12 pairs of bracts), *ca.* 320–380 μ wide; bracts strongly concave, subequally bilobed, with 1–2 antheridia each; bracteoles 1–2 at base of androecium. Gynoecia most frequently on short lateral branches, but *often on elongate leafy branches*; always with one subfloral innovation (rarely with a second one present). Female bracts exceedingly variable, always somewhat *reduced in size*, the *lobes lingulate to obovate* (75–260 μ wide × 340–460 μ long, occasionally 150 μ wide and only 275 μ long), crenulate on the margins, rounded to obtuse or subacute at apex; *lobules reduced in size*, from a minimum of 20–30 × 160–200 μ up to 35–50 × 170–230 μ occasionally 35–50 × 170–230 (270) μ, *varying from narrowly lingulate to linear* (mostly only 2–3, occasionally 4–5 cells wide), *acute to somewhat obtuse at apex, free for 0.4–0.75 of their length*. Bracteoles as large as or larger than bracts, (140) 190–245 μ wide × (275) 360–430 μ long, oblong-obovate, bifid for 0.3–0.5 of their length by a closed and sharp sinus, the *erect lobes acute*. Perianth at maturity usually *exserted for 0.6–0.7 of its length, obovoid*, 300–360 μ wide × 620–640 μ long, 5-carinate in the distal 0.3–0.4 of its length, the carinae nearly equal, the perianth *not compressed*; keels somewhat dilated above, somewhat *crenulate, the perianth-apex truncate*, but *beak not recessed;* beak short, formed of little elongated cells, *ca.* 9–11 × 23–25 μ.

TYPE: Mathesson Hammock, Old Cutler Road, s. of Coral Gables, Dade Co., Florida, on a decaying shaded log in deep hammock forest (*RMS 33860*, Dec. 27, 1954). Type collection in herbarium of author, and of Duke University.

DISTRIBUTION.—Known only from type collection.

DIFFERENTIATION.—*L. glaucescens* var. *obsoleta* is characterized by the dull texture, the densely chlorophyllose, elongated and ovate-pointed leaves (Fig. XXVII: 1, 4), the variability in position of both androecia and gynoecia (Fig. XXXVII: 1), the segmented oil-bodies (Fig. XXXVI: 6), and the similarity in facies to *L. glaucescens*. It may possibly prove to be a discrete species, and shares with *L. setiloba* a distressingly large number of features, among them (a) the high incidence of obsolete lobules, some of which, at least, bear 2-celled apical teeth (Figs. XXXVII: 1–2, 4–5; XXXVIII: 7, 11–12); (b) the occurrence of female inflorescences on both short lateral, and elongate leafy shoots (Fig. XXXVII:

1); (c) moderate sized, leptodermous cells, 17–20 (23) μ on the margins of most mature leaves; (d) bracts with small, but distinct lobules, acute and usually free for half or more of their length (Fig. XXXVIII: 1–2, 4–6); (e) bracteole bifid ⅓–½, with acute lobes; (f) underleaves often somewhat longer than broad, bifid fully to the middle or deeper (Figs. XXXVII: 1–3; XXXVIII: 8–10), the lobes 4, rarely 5, cells wide at base, most frequently terminated by 2 superimposed cells, the lateral margins occasionally angulate or obtusely unidentate; (g) perianth obovoid, scarcely compressed (Figs. XXXVII: 1; XXXVIII: 7).

The plants here tentatively designated as *L. glaucescens* var. *obsoleta*, diverge, however, from *L. setiloba* in a number of important regards. These differences suggest that the var. *obsoleta*, in spite of the obvious similarities in form of the reduced lobules, is not conspecific with *L. setiloba*. Among these divergent features are: (a) the more narrowly ovate-pointed leaves; (b) almost uniformly obsolete lobules, with no distinctly inflated ones present; (c) the reduced lobules with the apical tooth 1-celled, or equally frequently 2-celled—and apparently never 3–4-celled; the nearly typical plants of *L. setiloba* collected by Welch in Cuba, however, also have the vestigial lobuli commonly armed with an apical tooth only 2, rarely 3, cells long; indeed, in some instances the apical tooth is 1-celled; (d) androecia often terminal on long, leafy, leading branches, and then of 4–8 pairs of bracts; (e) female inflorescences with bracts shorter—usually much shorter—than vegetative leaves, with the lobule usually quite reduced in size. The differences in form of the bracts, of the vegetative leaves, and of the apical teeth of the reduced lobules are striking.

As is evident from the preceding, the variety *obsoleta* has reduced lobules with the apical teeth formed, in many cases, of a single projecting cell (terminated by the hyaline papillae, which disappear with age). On the basis of such lobules, the plants might be confused with such species as *L. minutiloba*. However, occasional to frequent leaves of the var. *obsoleta* possess a prominent apical tooth formed of two superimpsoed cells (Figs. XXXVII: 2, 5; XXXVIII: 11–12). Such apical teeth rarely if ever occur in any of our other species of the genus, and consequently are diagnostic.

In the somewhat longer-than-wide underleaves, with the lobes usually 4 cells wide at base, terminated most often by 2 superimposed cells, and in the often laterally angulate-unidentate margins, *L. glaucescens* var. *obsoleta* closely approaches *L. longifissa*. It agrees with that species also in the variable position of the female inflorescences, and, apparently, in the highly variable bracts, as well as in cell-size and polymorphic lobules. However, *L. longifissa* has a flattened, obcordate perianth, distinctly collenchymatous cells of both leaves and perianth, and quite differently formed reduced lobules of the vegetative leaves.

The size of robust plants of var. *obsoleta*, the dull texture when dry, the markedly ovate-pointed leaves, the long-exserted perianths (which are, furthermore, truncate at the apex), and the reduced size as well as form of the female bracts closely suggest typical *L. glaucescens*. Indeed, the facies of the two plants may be overwhelmingly similar. However, *L. glaucescens* var. *obsoleta* appears to differ consistently from typical *L. glaucescens* in the following points: (a) obsolete

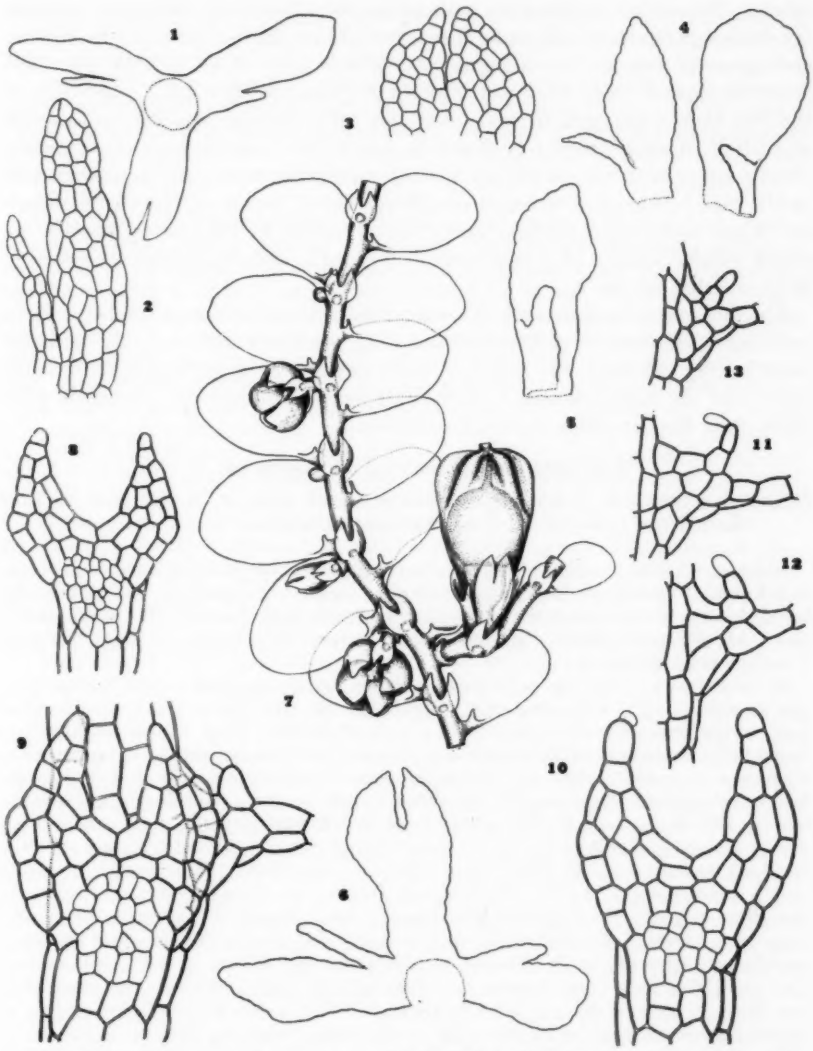


FIG. XXXVIII. *Lejeunea glaucescens* var. *obsoleta* Schust. 1. Bracts and bracteole, from cladogynous inflorescence (×65); 2. Single bract from Fig. 1 (×135); 3. Apex of bracteole (×135); 4–5. Bracts from acrogynous inflorescences (×65); 6. Bracts and bracteole (×65); 7. Fertile plant, with cladogynous gynoecia (×40); 8. Underleaf (×150); 9–10. Underleaves (×285); 11–12. Typical lobules (×150); 13. Lobule of *L. setiloba* to show comparative form (×150). (Figs. 1–12, *L. glaucescens obsoleta*, from type, *Schuster 36880*; Fig. 13, *L. setiloba*, from Cuba, *Welch*).

lobules; (b) smaller underleaves, with lobes only 4, rarely 5, cells broad at base; (c) deeper green, more chlorophyllose color; (d) the high incidence of androecia and gynoecia terminal on elongate leafy shoots (Fig. XXXVII: 1). This last criterion appears to be the most important character affecting a separation of the two plants, although it surely does not suffice to separate the var. *obsoleta* as a wholly discrete species. It should be noted that *L. glaucescens* var. *acrogyna* closely agrees with var. *obsoleta* in having occasional "terminal" androecia, and in the high incidence of acrogynous inflorescences. However, the var. *acrogyna* has larger underleaves, is light green like "typical" *L. glaucescens*, has the reduced lobules usually of a larger number of cells, and has a high incidence of large, inflated lobules.

The dull texture and densely chlorophyllose cells, and reduced lobules, suggest such autoecious species of *Rectolejeunea* as *R. phyllobola*. However, the perianth form is very different, the cells are much larger and never develop thickened walls, and the leaves possess a much more ovate-pointed form. Confusion with other than the preceding species is unlikely.

Lejeunea glaucescens var. **acrogyna** var. n.

Lejeunea caroliniana Schuster, *in* Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: 48, pl. X: 4–5, 1954 (not of Austin).

Formae typicae similis sed androeciis et gynoeciis saepe terminalibus in caulibus longis ducentibus; lobulis polymorphis saepe fere obsoletis, iis indistinctis haud dente apicali terminale praeditis. Specimen typicum legit R. M. Schuster et O. M. Schuster 28667, "Raven Rock," prope "Lillington, Harnett Co.," in Carolina Septentrionali.

Plants *dull, in light to pure green patches* on damp rocks. Mature (fertile) *plants 1.0–1.2 (1.4–1.5) mm. wide*. Stems *ca.* 90–100–150 μ thick; the ventral rows of cortical cells *averaging 30–35 μ wide* × 40–60 μ long. Plants slightly and irregularly branched; with 1 subfloral innovation (*exceptionally 2*). Leaves contiguous to weakly imbricate, *distinctly ovate, narrowly rounded distally*, nearly flat, varying from 560 μ long × 450 μ wide to 620 × 420 μ, to a maximum of 650–670 × 470–485 μ, rarely 700–850 μ long × 500–600 μ wide; keel exceedingly short, the posterior leaf margin nearly straight, not notched at apex of keel, the keel usually from 80–120 μ long when well-developed; lobule often vestigial and 2–3 cells long × 2 cells wide, tipped often by an elongate cell terminating in the slime papilla, varying to 120 μ long × 80 μ (rarely 125 μ) wide (4–5 cells long × 4, rarely 5–7 cells wide); apex a single cell usually, terminated by slime papilla; occasionally with inflated lobules up to 160 μ long × 140 μ wide, with keel *ca.* 150 μ long (*such lobules less than ¼ length of the dorsal lobe*, their area less than ¼ that of the adjacent underleaves). Underleaves averaging nearly to somewhat more than twice as wide as the stem, varying from a minimum of 170 μ wide × long to *ca.* 200 μ wide × 220 μ long, rarely up to 220 μ wide × 225 μ long (averaging almost or quite as wide as long, but *never wider than long*), bilobed for ½ their length, the lobes ovate-triangular, *usually 6–8 cells wide at base*; sinus often narrowly lunate, if narrow, at least rounded below; underleaf-bases often with a discrete "disk" formed of 16–25, rarely more, small, potential rhizoid-initials (which, however, rarely form rhizoids). Cells of margin, *at leaf-apex (17) 18–21* μ, quadrate, median cells somewhat rectangular to polygonal, averaging (19) 22–26 μ wide x (23) 28–32 μ long (but rarely locally, in some leaves only 17–23 μ wide); cell-walls thin throughout, the trigones small

to very small, concave; without intermediate thickenings. Each cell typically with *9–18 relatively obvious oil-bodies* (in the marginal and submarginal cells often much smaller, and only 5–8 per cell), the oil-bodies usually appearing almost to fill the cell-lumen; oil-bodies of median cells varying from spherical and 3, but usually 4–6 μ, to ellipsoidal and 5 × 6–9 μ or 4 × 10 μ, formed of obviously individually protruding globules—thus *clearly segmented*; the individual globules varying from 1 to 2 μ, but nearly of equal size in any one individual oil-body. Chloroplasts averaging considerably smaller than oil-bodies, up to 3 × 4 μ; cuticle smooth.

Autoecious. Male inflorescences *indiscriminately developed on elongate (main) axes or on short lateral branches*, of 4–5 (6) pairs of bracts; bracts imbricate, becoming progressively smaller distally, forming a short spike, 2-lobed, strongly ventricose, with a strongly arched keel; bracteoles at base of spike only, 1 or 2, up to 180 μ long x wide. Female inflorescence *indiscriminately on an elongate, main axis, or on a short lateral one* (which innovates almost immediately on one side); inflorescences of main axes almost invariably with one innovation on one side, causing the inflorescence to become pseudolateral, occasionally *with two equal innovations* (with the inflorescence then in notch between them). *Bracts* (of acrogynous inflorescences) *large*, with lobes 550–650 μ long × 250–350 μ wide, occasionally up to 825 μ long × 420 μ wide, narrowly lingulate to obovate, *nearly or quite as long as adjacent leaves*; *lobules vestigial*, short flaps 150–250 μ long (averaging no more than ⅓ the lobes in length) × 30–65 μ wide, the distal ⅓–½ free, *forming often a linear appendix only 1–2 cells wide*, terminated by a slime papilla; bracts and bracteole only very slightly or not at all united at base; bracteole from *ca.* 200 μ wide × 360 μ long to 240 μ wide × 400 μ long, occasionally 350 μ wide × 550 μ long, narrowly obovate, bilobed *ca.* ⅓, the *lobes subacute to acute*. Mature perianth (of acrogynous inflorescences) *scarcely or not exserted* beyond the bracts (when sporophyte half-grown), oblong-obovate, but without wings widest at or below middle and ellipsoidal in outline, *ca.* 850 μ long × 350 μ wide (without wings; with wings 450 μ wide), not at all dorsiventrally compressed, the antical keel distinct, the postical as sharply developed as the lateral, the keels descending about 0.35–0.45 of the perianth-length; keels wing-like, dilated, especially above (the maximal perianth width thus just before the sharply and swiftly contracted apex), the margins distinctly crenulate by projecting cells; apex subtruncate, with a short beak, set at times in a very slight recession; apical cells of beak *ca.* 30–34 × 10–13 μ.

TYPE: On shaded damp rock, Raven Rock, Cape Fear R. *ca.* 6 mi. n. w. of Lillington, Harnett Co., North Carolina, July 19, 1953 (*R. M. and O. M. Schuster 28667*).

Known only from the outer edge of the Piedmont of North Carolina (Raven Rock, on Cape Fear R., *ca.* 6 mi. n. w. of Lillington, Harnett Co., *Schuster* and *Schuster 28667*, July 19, 1953). A second collection of sparse material from the same locality, October 1955 (*RMS 37401*).

The single known occurrence of the variety is from a shaded, damp ledge, adjacent to an intermittent stream which cuts through an elevated bluff. Associated are *Radula obconica*, *Porella pinnata*, *Plagiochila yokogurensis* subsp. *fragilifolia*, *P. asplenioides*.

VARIATION AND DIFFERENTIATION.—This taxon is characterized by the often vestigial lobules—most of which attain only 100 μ in length × 50–60 μ in width. In the possession of polymorphic, often poorly developed lobules it closely agrees with several other *Lejeunea* species known from the tropics and subtropics, among them *L. minutiloba*, *cladogyna*, and *floridana*. The present taxon has

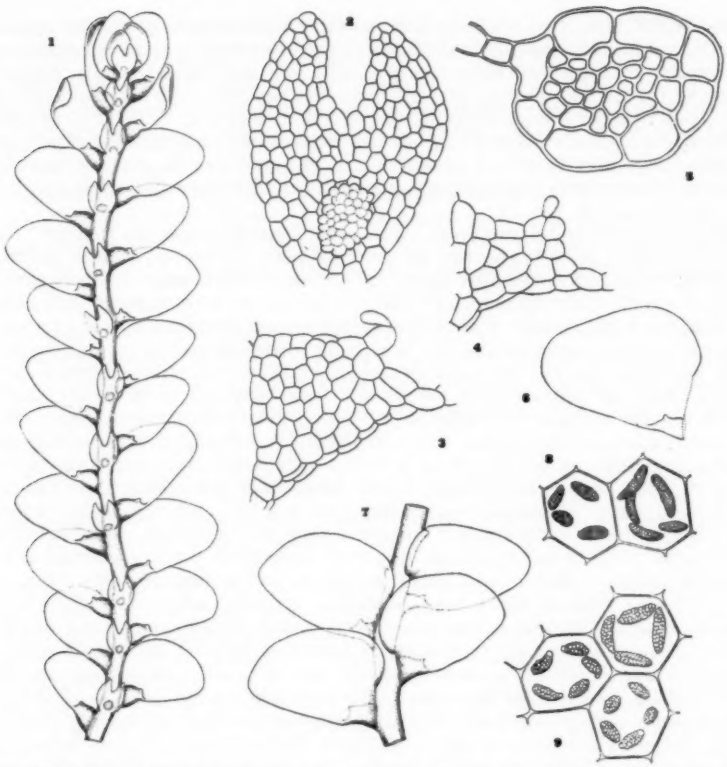


FIG. XXXIX. *Lejeunea glaucescens* var. *acrogyna* Schust. 1. Sterile shoot, of extreme with maximal development of lobules (×30); 2. Normal underleaf (×125); 3–4. Two lobuli, showing variation in size (×200); 5. Cross-section of stem (×275); 6. Optimal leaf (×43); 7. Shoot-sector, antical view (×43); 8–9. Cells with oil-bodies (8, ×420; 9, ×417). (All drawn from type material, from Raven Rock, Harnett Co., North Carolina).

very often acrogynous inflorescences with large female bracts (at least 550–650 μ long × 250–350 μ wide), which are nearly or quite as long as adjacent leaves (the leaves average up to 650–700 μ long); the lobules of the bracts are extremely reduced, to a short flap (150–250 μ long), average at best one-third the length of the lobes, and are very narrow; on one side they may be free for half their length, the free portion attenuate and only 1–2 cells wide except at its juncture with keel. In the often acrogynous gynoecia and large size of the bracts, with their lobules extremely small, the plant differs at once from *L. cladogyna* (where the bracts are mostly 280–350 μ long, with the lobules at least two-thirds the length of the bracts). Inversely, the partly acrogynous gynoecia and the large size of the bracts ally this taxon with *L. minutiloba* and *floridana*.

Lejeunea floridana differs from *L. glaucescens* var. *acrogyna* in the larger,

pellucid cells of the cortex of the stem; in the broader-than-long underleaves; in the merely retuse or emarginate bracteole and in the even more strongly reduced lobules of the bracts. Only *L. minutiloba* remains to be separated from *L. glaucescens* var. *acrogyna*. The present plants are much larger: 1–1.4(1.5) mm. wide, with the dorsal lobes up to 700–850 μ long (plants up to 850 μ wide, dorsal lobes up to 500 μ long in *L. minutiloba*). There are also differences in cell size. In *L. glaucescens* var. *acrogyna* the marginal cells average 17–20 μ near the leaf-apex (13–16 μ in *L. minutiloba*). In *L. glaucescens* var. *acrogyna* the bracts have narrowly lingulate lobes, in *L. minutiloba* they are obovate to broadly lingulate. Finally, the lobules of the bracts appear even more highly reduced in *L. glaucescens* var. *acrogyna* than in *L. minutiloba*: one (or both) of each pair of bracteal lobules is free for about one-half its length, the free portion linear and only 1–2 cells wide; in *L. minutiloba* the free portion of the lobule is about 60 μ wide and 3–4 cells broad, usually rounded distally and lingulate—rather than linear. The segmented oil-bodies, of living plants, of course, immediately distinguish *L. glaucescens* (and var. *acrogyna*) from all phases of *L. minutiloba*.

Several peculiarities of *L. glaucescens* var. *acrogyna* may be further emphasized. One is the large size of the oil-bodies, which, collectively, at times obscure much of the cell-lumen, making it relatively opaque (compared with our other species). In part of the type collection, the median cells may have very many oil-bodies (usually 9–18), nearly filling the lumen. However, other plants show only 2–5 much larger oil-bodies per cell. These plants, otherwise almost identical in appearance, seem to belong to the same population. The one difference noted was that the type with many oil-bodies was somewhat less robust (up to 1 mm. wide) and had uniformly uninflated lobules; by contrast the phase with large oil-bodies, few per cell, was up to 1.4 mm. wide and frequently had inflated lobules.

Another peculiarity is the occasional or rare development of a subfloral innovation on each side of the inflorescence (which then comes to lie in the angle between the two innovations, and remains terminal in appearance—rather than becoming pseudolateral, as is normally the case). Further deserving emphasis is the fact that the female inflorescence often appears terminal on an elongate shoot—at other times on a short branch. The male inflorescence is terminal on either a long or a short shoot. In some cases a single elongate shoot will develop 4–5 small lateral androecial branches (definitive in length, and with usually only 2–3 pairs of bracts), which may be briefly separated; in other cases larger androecia (with 3–5, rarely 6–9 pairs of bracts) are found, and these are produced more sparingly; either they are terminal on main shoots, or they occupy short, lateral shoots.

These last peculiarities of the var. *acrogyna* suggested that a species wholly distinct from *L. glaucescens* was at hand. However, the leaf-shape, polymorphism in lobule form (and small maximal size of the lobule), the underleaf shape and size, perianth form, and the oil-bodies all suggest *L. glaucescens*. Evans (1905, p. 188) explicitly states that *L. glaucescens* has a single subfloral innovation, and bracts that are shorter than the leaves. He also (Evans, 1918, p. 136) states that

"the female branch seems to be constantly very short, just as in *L. cladogyna*." On that basis, the var. *acrogyna* was originally held to be an independent new species. However, as has been previously emphasized, study of a very long series of specimens has shown that *L. glaucescens* may have occasional acrogynous inflorescences (although none of these that have been noted bear more than one innovation), and such inflorescences have larger bracts than the normal, cladogynous inflorescences.

Study of the somewhat more copious plants of var. *acrogyna* collected in 1955 narrow down the differences between the two entities. The latter collection is of plants with general development of lobulate leaves, in which the gynoecia are usually cladogynous, but not exclusively so; the androecia always on short lateral branches (and in some cases forming elongate spikes, of 6–7 or even 8–9 pairs of bracts!); the innovations beneath the gynoecia almost always single. As a consequence, var. *acrogyna* is maintained as distinct largely on the basis of (a) somewhat smaller marginal cells, 17–20 μ in tangential diameter; (b) frequent acrogynous inflorescences; (c) androecia sometimes on leading, leafy branches; (d) female bracts with free apices of lobules generally reduced to an obsolete tooth 1–2 cells broad; (e) perianths slightly to hardly emergent from beyond the bracts. The geographical isolation suggests that perhaps treatment as a subspecies may eventually prove desirable, although this is presently premature.

Lejeunea calcicola sp. n.

L. glaucescenti similis sed differt gynoeciis saepissime in caulibus foliaceis ducentibus; amphigastriis late rotundatis, sensim latioribus quam longioribus, basi rotundatis; foliis plerumque dimidiate ovatis, apice obtusis vel apiculatis; perianthiis carinatis, carinis aspectu crenulatis de causa cellularum gibbosarum projicientium. Specimen typicum legit R. M. Schuster, no. 33321, "Aspalaga Bluff," prope "Appalachicola R., Gadsen Co., Florida."

Plants scattered over damp, shaded, calcareous soil or coral, or growing over *Dumortiera*, rarely in small, *dull to scarcely shiny*, yellow-green patches. Mature sterile shoots 900–1200 (rarely 1350–1450) μ wide, on fertile branches often narrower. Stems 80–100 (120) μ in diam., slender; ventral cortical cells averaging 30–36 μ wide × 45–65 μ long. Plants usually *very freely branched*, with consistently one subfloral innovation. Leaves distant to weakly imbricate, from asymmetrically subovate to (*near female inflorescences*) *dimidiate-ovate*, narrowly rounded distally to *obtusely to weakly pointed, often somewhat repand* (especially near female inflorescences), but on sterile shoots entire, slightly convex, especially in drying, from 560–720 μ long × 560–650 μ wide, occasionally up to 670–740 μ long × 670–700 μ wide; antical margin *somewhat concave or nearly straight and parallel to stem basally, suddenly strongly convexly arched, appearing broadly dilated*, above base arching outward towards the narrowly rounded to *obtusely pointed apex*; postical margin weakly arched or nearly straight, except distally where curved into the apex, *not or obsoletely notched at juncture with keel*; keel exceedingly *short, on fertile plants very largely vestigial*, to a maximum of 150 μ when well-developed. *Lobule usually vestigial*, but on sterile shoots occasionally well-developed; when vestigial only 2–3 cells long × 2 cells wide; when inflated up to 4–6 cells long × 5–7 cells wide, with involute free margin, then up to 140 μ long × 100 μ wide (*up to 0.2–0.24 of the length of dorsal lobe; its area up to 0.4–0.5 of that of the underleaves*). Underleaves averaging 1.5–2 times the stem width, rarely slightly wider, on main stems up to *210–250* μ

wide × *180–200 μ long* (*width 1.15–1.25* [*1.35*] *times the length*), bilobed for slightly over half their length, the sinus sharp and acute, the lobes acute to subacute, their lateral contours evenly rounded; *lobes 4–5, occasionally 6 cells wide at base*, terminated usually by a solitary cell; rhizoid-bearing disk poorly developed, of few cells (usually fewer than 16), usually developing rhizoids. Cells of the margin and of leaf-apex (of mature leaves) *20–23* (*25*) *μ*, quadrate or subquadrate; median cells rectangular to hexagonal, averaging *24–30 μ wide* × *34–40 μ long;* cell walls exceedingly thin, without intermediate thickenings, but *with minute trigones;* oil-bodies few per cell, fine-segmented or papillose, from 5 × 6 *μ* to 4 × 7–10 *μ* up to 5 × 11 *μ*.

Autoecious. Androecia *compactly spicate, sessile on short lateral branches* of determinate length, without the ability to proliferate vegetatively, *elongate*, 280–300 *μ* wide, of *4–8 occasionally 10–12 pairs of closely imbricate bracts;* bracteoles small, bifid, 1–2 at base of androecial spike. Female inflorescence indiscriminately on a *long primary or secondary branch, or at apex of a short, weak lateral branch* bearing only 1–2 rudimentary basal leaves; always with a single subfloral innovation. Inflorescences of long leafy branches more robust; subtending 1–3 pairs of leaves, dimidiate-ovate and often repand, usually obtusely pointed at tip; *bracts with lobe as long as or longer than leaves*, narrowly oblong to obovate or lingulate, up to *750–800 μ long* × 250–320, occasionally 360 *μ* wide, sinuate to repand on the margins, occasionally weakly pointed; *lobules very small to vestigial*, merely 200–300 (340) *μ* long × 30–50, rarely 60 *μ* wide (thus a mere basal fold only 0.2–0.4 of the length of lobe, free for ¼–½ its length); *bracts essentially or quite free from bracteole*; bracteole obovate, 520–560 *μ* long × 350–380 *μ* wide, *bilobed 0.3–0.4 of its length*, the sinus sharp and acute, often closed, the erect to connivent *lobes acute or subacute*. Inflorescences of short lateral branches much smaller (bracts with lobes up to 500 *μ* long; usually distinctly shorter than leaves of main shoots). Perianth oblong, sharply 5-carinate to the middle, not or barely compressed, widest (exclusive of keels) at the middle, 330–450 *μ* wide × 700–760 *μ* long, with age stipitate and quite longly exserted (0.5–0.6 of its length exserted beyond bracts then), the *keels strongly crenulate* due to bulging gibbous cells, *not produced distally*, the *beak rather long and prominent*, 60–70 *μ* long, not recessed.

TYPE: Over calcareous damp soil of bank, and over *Dumortiera hirsuta*, Aspalaga Bluff, along Appalachicola R., Gadsden Co., Florida (*RMS 33321*).

DISTRIBUTION.—Known only from the type, and two other collections from Florida: "Buzzard's Roost", *ca.* 7 mi. w. of Gainesville, Alachua Co. (*RMS 33348*); Pronto Springs, *ca* 22 mi. n. of Tallahassee, Gadsden Co. (*Redfearn 1255*!).

The Alachua County plants differ somewhat from the type material, chiefly in: (a) the consistently smaller androecial spikes, formed of only 3–5 pairs of bracts; (b) the sometimes slightly larger underleaves, up to 270 *μ* wide × 220 *μ* long, with the lobes up to 6–8 cells broad; (c) the occasionally less sharply constricted leaf-bases. Both collections agree in the high incidence of obovate, sharply basally narrowed leaves on immature stems. Such obovate leaves are relatively rare in the sparse material collected by Redfearn. These plants, however, show the typical, smooth-margined, broad underleaves of the species (up to 230 *μ* wide × 190 *μ* long), and the very characteristic tendency for many leaves, especially on fertile plants, to be sharply apiculate (occasionally with a point formed of 2 superimposed cells) or bluntly pointed. They also show the

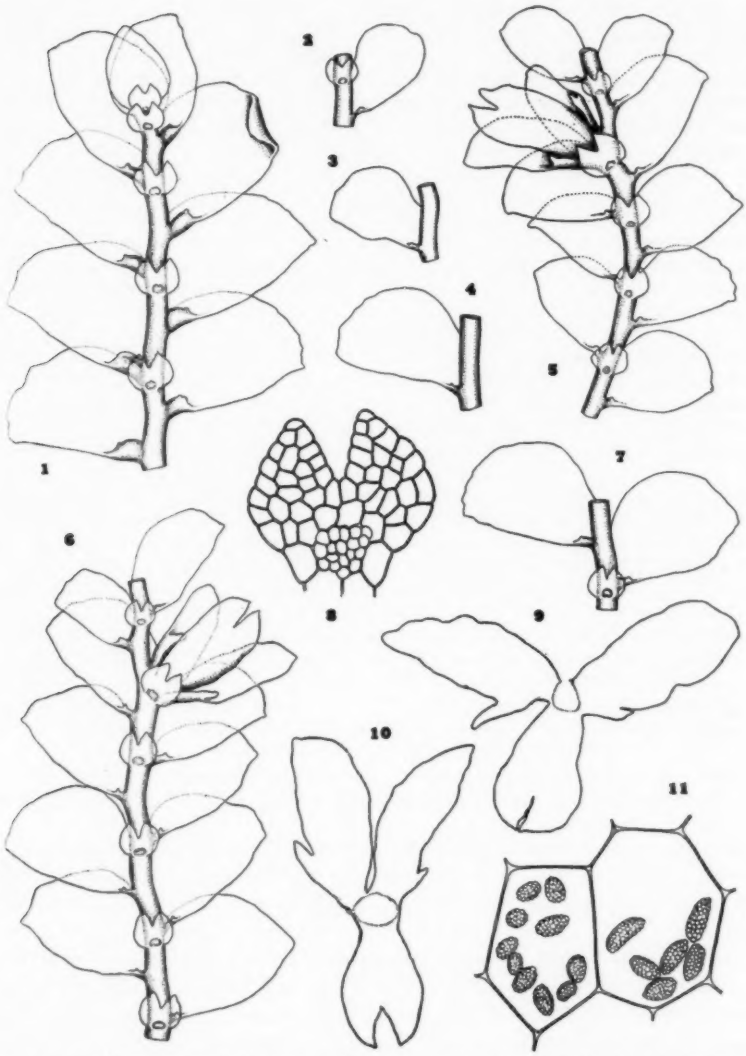


FIG. XL. *Lejeunea calcicola* Schust. 1. Robust sterile shoot (×33); 2–4, 7. Sectors of sterile shoots with more or less juvenile, obovate leaves (×29); 5–6. Fertile shoots, with acrogynous gynoecia (×33); 8. Underleaf (×175); 9–10. Acrogynous bracts and bracteoles (×33); 11. Median cells, with oil-bodies (×660). (All from type, Aspalaga Bluff, Florida).

very diagnostic female bracts, with narrow lobes, exceeding the leaves, but vestigial lobules.

ECOLOGY.—Both of the writer's collections from over, and amidst, *Dumortiera hirsuta*. At the type locality also with *Lophocolea cf. bidentata*, *Mnium*, and

other mosses, on shaded calcareous soil. At the Gainesville locality over coral limestone rock-walls, associated with *Cololejeunea ornata* and *contractiloba*, and in part admixed with *Lejeunea minutiloba* (which may be distressingly similar, except for the oil-bodies). The calcicolous habitat perhaps distinctive. At all three known localities mixed with, or growing adjacent to, *Crossotolejeunea bermudiana*.

DIFFERENTIATION.—This problematical species shows similarities to several regional species, chief among them *L. floridana* Evs. It shares with this latter species fundamentally similar bracts, free from the bracteole, with similarly reduced and flap-like lobules (Fig. XL: 9–10). However, the bracteole in *L. calcicola* is uniformly sharply bilobed, with the lobes acute or subacute (in *L. floridana* merely shallowly emarginate with vestigial, rounded lobes). The two species also share underleaves that are almost constantly conspicuously broader than long (in which feature they differ from our other species of *Lejeunea*); they have similarly slightly crenulate leaf-margins; they possess in common a strong tendency for the lobules to be reduced or vestigial (Fig. XL: 2–7), and also almost equally large cells and similar oil-bodies (Fig. XL: 11); the spicate androecia, often formed of many pairs of bracts, are strikingly similar. In addition to the differences in the bracteoles, *L. calcicola* differs from *L. floridana* as follows: (a) perianths with keels not produced distally (Fig. XLI: 6–7), thus with the beak not appearing recessed; (b) in *L. calcicola* the leaves, especially of less robust shoots, are constricted near the antical base, and somewhat asymmetrically obovate at times (Fig. XL: 2–4); (c) the underleaves average smaller in *L. calcicola* and never appear to develop a distinct "disk" of numerous small, potentially rhizoidous cells (Figs. XL: 6–8; XLI: 3–4).

The form of the immature leaves (Figs. XL: 2–4, 7; XLI: 1) is to be regarded as one of the most important features of *L. calcicola*. They are at times reminiscent, in this feature, of the leaves of *Prionolejeunea aemula*, although this latter species shows an even more pronounced and abrupt dilation of the dorsal lobe somewhat above the antical base, resulting in a dorsal lobe that appears to be constricted near its antical base. In *L. calcicola* the mature leaves are broad (width sometimes almost equal to length), and show this feature much less distinctly. Even in the mature leaves, however, the margin above the antical base is straight for some distance, lying parallel to the stem, and then suddenly arches outward, resulting in a lobe which appears strongly dilated some distance above the narrowed antical base.

Another distinctive feature of *L. calcicola* is the large cell size (the marginal cells varying from 20–25 μ; the median up to 26–30 $\times$ 35–38 μ in mature leaves). In the large cells the species agrees with or approaches *L. glaucescens* and *L. floridana*, but differs from *L. cladogyna* and *L. minutiloba*, which have smaller cells.

The great degree of variation in length (and robustness) of the female branches is quite characteristic of this species. In some cases these branches are all very short, and arise from branches of the second or third order, from a highly ramified branch system (Figs. XLI: 6–7); in such cases the bracts are small, often only 450–550 μ long, and may sheath less than the basal half of the relatively

small perianths, much as in *L. glaucescens*.[34] In other cases the perianth is at the apex of long leafy shoots; the bracts then are very large (up to 800 μ long) and commonly exceed the leaves in length (Fig. XL: 5–6). In this high degree of variation in position of the female inflorescences *L. calcicola* closely agrees with *L. minutiloba*.

Lejeunea calcicola differs from *L. minutiloba* in the few and segmented oil-bodies, in the larger cells, and in the form of mature leaves. However, it agrees with *L. glaucescens* in the oil-body type, and its distinction from this species is tenuous at times. The two species share the tendency for development of ovate to almost dimidiate-ovate leaves (on mature stems); the dull to barely glistening color, when dry; a similar perianth-form. Furthermore, *L. glaucescens* may occasionally have underleaves that average fully as wide as long, with occasional ones slightly broader than long. However, the basally rounded to subcordate underleaves of *L. calcicola*, uniformly broader than long, are distinctive. *L. calcicola* also has a much higher incidence of reduced-lobulate leaves, a much greater variability in position of the female inflorescences; and an even greater variability in the female bracts, some of them being considerably longer than vegetative leaves. I have seen no plants of *L. glaucescens* in which the leaves are bluntly or sharply pointed, and have seen none with obovate juvenile leaves.

In Mexico, and perhaps elsewhere, occurs a plant provisionally referred to *L. calcicola*, which closely approaches *L. floridana* in some respects, but differs in several major points; this the writer would separate as:

Lejeunea calcicola var. **mexicana** var. n.

Lejeunea calcicolae typicae similis sed L. floridanam adducens bracteis lobulis obsoletis; bracteola obsolete biloba; foliis sine lobulis vel cum lobulis fere obsoletis; cellulis corticalibus maximis 40 μ latis. Specimen typicum legit R. L. McGregor, no. 8011, "Choy Spring," prope "Valles, San Luis Potosi, Mexico."

Similar to *L. floridana* in size and facies; shoots up to 1425 μ wide; plants yellowish-green or green in drying, dull; *stem with ventral cortical cells 35–38, rarely 40 μ wide even on robust stems*; underleaves broader than long (usually 160 μ long × 180–200 μ wide) but *occasionally as long as broad* (up to 220 × 220 μ), *or longer than broad* (190 μ long × 185 μ wide), *at base rounded to somewhat cuneate*, crenulate on margins; cells as in *L. floridana*, with segmented oil-bodies. Female inflorescences indiscriminately on either short lateral shoots, or leading, leafy shoots, with one subfloral innovation which is usually again fertile Bracts as in *L. floridana*, the lobule usually vestigial and united for all or almost its entire length with lobe; *bracteole shortly bifid, usually for 0.15–0.25 of its length, the lobes acute, erect or connivent, often terminated by 2 superimposed cells*,

[34] This condition is typical of the collection from Alachua Co., Florida (*Schuster 33348*), as is clear from Fig. XLI: 6–7. In this case the gynoecia are virtually all clearly cladogynous, with most female branches very short. In such cases both bracts and perianths may be very short, and in some cases (Fig. XLI: 7) the gynoecial innovations may be either absent or remain as a rudimentary Anlage! Such is the condition typical of *Potamolejeunea*, suggesting again that the latter is an artificial group. When innovations are produced these are often short and sterile; in one instance an innovation was produced clearly from the adaxial side of the cladogynous branch, rather than from the more normal, abaxial side (Fig. XLI: 6).

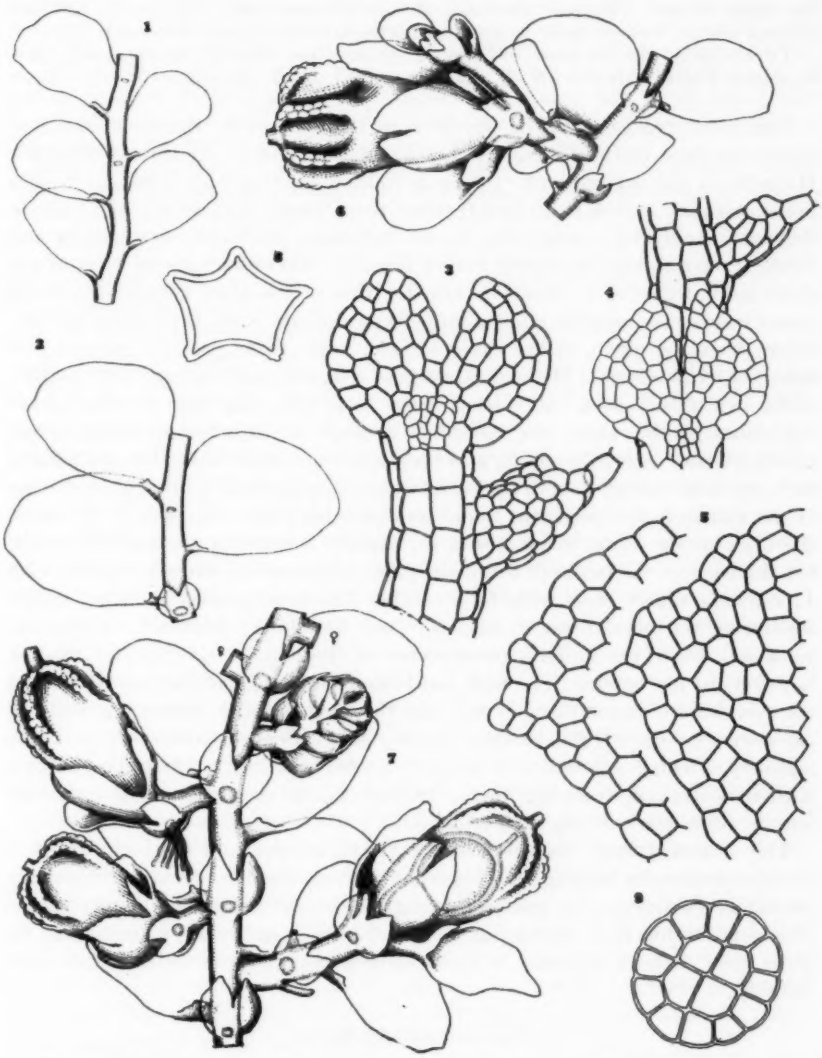


FIG. XLI. *Lejeunea calcicola* Schust. 1. Sector of sterile, juvenile shoot (×37); 2. Sector of sterile, mature shoot (×37); 3–4. Lobules and underleaves, *in situ* (×156); 5. Two lobe apices, showing maximal degree of development of apiculi (×200); 6–7. Shoot sectors with gynoecia on short lateral branches (×43); 8. Cross-section of perianth in upper one-third (×68); 9. Cross-section of seta (×165). (Figs. 1–5 from type, Aspalaga Bluff, Florida; 6–9, from *Schuster 33348*, Alachua Co., Florida).

the sinus closed. Perianth obovoid, the distal one-third with keels that are dilated above, but scarcely project as horns, the short beak recessed.

TYPE: Moist shaded bank of Choy R., below Choy Spring, 10 mi. e. of Valles, San Luis Potosi, Mexico (*R. L. McGregor 8011*, as *L. cladogyna*).

This variant differs from *L. floridana* in the characters which are italicized above. In these features it approaches *L. calcicola* and *L. glaucescens acrogyna*. However, it differs from both of these as follows: (a) bracts with lobules usually more vestigial, united for 0.75–1.0 times their length with lobe; (b) bracteole divided for only $\frac{1}{5}$, occasionally $\frac{1}{4}$, of its length; (c) leaves with lobules uniformly vestigial, formed of only a very few cells. These features all suggest the more mesic forms of *L. floridana*. On the basis of the often broader-than-long under leaves it is suggestively similar to typical *L. calcicola*. It differs from this, however, in the ovate, rather than obovate, form of the juvenile leaves (or of leaves of weak shoots). The ventral cortical cells also may average very slightly wider in extreme cases (on robust stems 40 μ wide). In spite of this, future collections of this plant may establish a range of variation showing perfect intergradation with *L. calcicola*, and necessitate uniting it with this. An affinity to *L. calcicola* (rather than to *L. floridana*) is also suggested by the fact that none of the relatively few perianths found showed a horn-like extension of the keels, the apex of the beak, in all instances, slightly surpassing the perianth-keels. On the basis of the perianth form, the plant consequently must be placed with *L. calcicola* rather than with *L. floridana*. The most readily evident feature separating the plant from *L. calcicola* (and the closely related *L. glaucescens acrogyna*) lies in the uniform obsolescence of the lobules; this may be related, however, to the habitat. The high incidence of underleaves that are as long as (or even barely longer than) broad, and the general facies, suggest strongly *L. glaucescens acrogyna*. From this the var. *mexicana* differs very obviously in (a) the majority of female inflorescences on short, lateral branches; (b) androecia always, without exception, forming short, leafless, lateral branches of determinate length; (c) absence of any inflated lobules.

The underleaf form, the obsolescence of the lobules, and the high incidence of inflorescences on leading leafy branches separate the plant from the habitually similar *L. glaucescens*. In spite of the original determination, the plant bears no close relationship to *L. cladogyna*. In the misleading key of Frye and Clark, the plant keys without difficulty to *L. minutiloba*, to which it also bears no relationship.

Lejeunea setiloba Spruce

Lejeunea (*Eu-Lejeunea*) *setiloba* Spruce, Trans. Edinburgh Bot. Soc. **15:** 281, 1884.
Lejeunea setiloba Evans, Mem. Torrey Bot. Club **8:** 148, 1902; Evans, The Bryologist **43:** 2, 1940.

Plants small to medium size, *pale green*, prostrate, 800–1000 μ wide. Stem 55–65 μ in diam.; *ventral cortical cells 20–26 × 35–45 μ*. Leaves oblong-ovate, 300–400 μ wide × 400–500 μ long, usually *broadly rounded distally, widest near middle*; lobules polymorphic: some normal and inflated, though small, others (or often almost all of them) vestigial, consisting of a basal

fold of a few cells, bearing a conspicuous apical tooth; *apical tooth of reduced lobules (1) 2–4 cells long*, parallel with stem or slightly divergent, bearing the hyaline papilla at its inner apex. Cells *ca.* (17) 18–20 μ on margins, *ca.* 22–24 × 23–26 μ medially, the median hexagonal, exceedingly leptodermous. Underleaves from *ca.* 150 μ long × 100–120 μ wide to 155–170 μ long × 150–155 μ wide, up to twice as broad as stem, bifid for 0.5–0.65 of their length, the acute *lobes usually 4*, less often 5 *cells wide at base, terminated most often by 2 superimposed cells, the lateral margins often angulidentate or angulate.*

Autoecious. Androecia on short, sessile lateral branches; bracts in 2–3 pairs. Female inflorescences on short lateral branches, or on *elongate leafy branches. Bracts subequal in length to larger leaves* (lobe 500–550 μ long × 260–325 μ wide, obovate to obovate-oblanceolate, rounded to obtuse at apex; *lobule subequal in length*, up to half as wide, acute); bracteole *ca.* 450–470 μ long × 200–260 μ wide, oblong-obovate, bilobed up to ½ its length, the approximated lobes acute. Perianth submersed, up to 650 μ long × 500 μ wide, scarcely compressed, *antical keel sharp.*

TYPE: Brazil, Manaos, near mouth of Negro River (*Spruce*).

DISTRIBUTION.—A tropical species, found from Brazil (type) northward to the West Indies (Cienfuegos, Soledad, Las Villas Prov., Cuba, *Welch 9392, 9367 p.p.*!). Up to the present not known from our area.

DISCUSSION.—*L. setiloba* is included in the present treatment because of the probability that it will eventually be found in the southern tip of Florida, and because material of *Lejeunea glaucescens* var. *obsoleta* and of *Rectolejeunea phyllobola* may be confused with it, leading to erroneous reports. Indeed, isolated gatherings of the last two species were initially referred to *L. setiloba* by the writer, although typical plants of the two taxa cannot be confused!

L. setiloba, in its size, and in the size of its cells, occupies almost a middle point between the small, and small-celled *L. minutiloba* and *L. cladogyna*, and the robust, larger-celled *L. glaucescens*. The less optimal versions of the latter may have cells of similar size (averaging 17–20 μ on the margins of the leaf-tips, *ca.* 20–24 μ wide medially). Indeed, the cells of *L. glaucescens* var. *obsoleta* and var. *acrogyna* almost perfectly match those of *L. setiloba* in size. As is developed under the var. *obsoleta*, these two taxa agree in other respects as well. Consequently, *L. setiloba* is to be separated with care from *L. glaucescens* and its varieties.

Two features allow its recognition from all phases of *L. glaucescens*. Firstly, the reduced lobules bear apical teeth which are mostly 2–4 cells long, and often diverge slightly from the stem (Fig. XXXVIII: 13). In *L. glaucescens*, the reduced lobules of the leaves, if present, bear short apical teeth formed of 1 or at most 2 cells, at whose tip a hyaline papilla may be situated. Secondly, in all phases of *L. glaucescens*, the female bracts are strongly, unequally bilobed, with the lobule greatly reduced in size, while typical *L. setiloba* has the lobule nearly subequal in length to the lobe.

Lejeunea setiloba can be separated from all of our other species of *Lejeunea* by the subulate apical tooth of at least some of the vestigial lobules. As Evans (1902) has emphasized, the type material showed both small inflated lobules, with the normal, one-celled apical tooth, and lobules which were reduced and bore a filiform apical tooth which (*fide* Evans, 1940) may be 1–4 cells long. The Cuban material is closely similar, although the apical teeth usually are two-celled. A striking peculiarity of the material seen is the sharp dimorphism in

lobule form. On some shoots almost all lobules are quite vestigial; on others all or the majority are large and inflated, with none intermediate in shape. In this respect, *L. setiloba* closely recalls *L. minutiloba* var. *heterogyna* and *L. cladogyna*.

A poorly developed, perianth-free Floridian collection was, for some time, referred to *L. setiloba*. These plants were determined and cited as *L. cladogyna* by Redfearn (1952), but after many hours of careful comparison I am now convinced that they are a very weak elobulate extreme of *Rectolejeunea phyllobola* with the following features that are suggestive of *L. setiloba*: shoots 660–975 μ wide, i.e., small and delicate; stems only 60–68 μ in diameter, with ventral cortical cells only (18) 20–25 μ wide; leaves oblong-ovate, from 340–355 μ wide $\times$ 425–460 μ long, up to a maximum of 450 μ wide $\times$ 500 μ long, but more clearly ovate than in the Cuban plants; lobules largely vestigial, and with apical tooth obliquely spreading, 2–4 cells long, with terminal hyaline papilla; underleaves 100–125 μ wide $\times$ 110–135 μ long, bifid for 0.5–0.65 of their length, the lobes 4, occasionally 5 (–6) cells wide at base, occasionally unidentate on sides, very narrow and often terminated by two superimposed cells; androecia on short lateral branches, with 2–3 pairs of bracts; gynoecia usually on short, rarely leading shoots. The obsolete or nearly obsolete lobules, with an elongate, obliquely spreading apical tooth 2–4 cells long serves to separate these plants from almost all other species known from our region.

Although the Floridian plants closely approach *L. setiloba* in the long tooth of elobulate leaves, the plants possess several deviant features. Among these are: marginal cells small, averaging 14.5–17 μ on the lobes; median cells often smaller and only 19–22 $\times$ 20–25 μ, but occasionally larger and up to 21–24 $\times$ 23–27 μ. More serious is the discrepancy in the form of the perichaetial bracts. In the Cuban plants of *L. setiloba* the lobules are very large and may nearly equal the lobes in length; in the Floridian collection the lobules are short, perhaps 0.4–0.5 of the length of the lobes. The distinctly inflated lobules of typical *L. setiloba* are never developed.

This material, which superficially is hardly determinable with certainty, is cited at length since it illustrates several salient points. The material was too fragmentary to refer safely to any species, although the cell-size, the lobule, underleaf and particularly the bracts (with their very small lobules) and bracteoles clearly eliminated *L. cladogyna* and pointed to *R. phyllobola*. Therefore this fragmentary collection should never have been utilized for publication; but having once been cited in the literature, it had to be disposed of elsewhere. In this process, the extraordinarily close affinities between *Rectolejeunea*, *Lejeunea* and "*Stylolejeunea*" were again forcibly brought home to the writer! The Redfearn material recalled the recent discussion by Arnell (1953) of *R. rhodesiae* (Sim) Arnell, one of the two species originally cited under *Stylolejeunea*. Arnell showed that this plant possessed variation from inflated lobules to a vestigial lobule with an apical tooth "to 5 cells long, frequently ending in a slime-papilla." The figures he gives of lobules of *R. rhodesiae* can be wholly matched if a series of collections of *R. phyllobola* are studied! Yet this identical variation from a "normal" inflated lobule with proximal hyaline papilla to a vestigial lobule with a slender, attenuate apical tooth terminating in a hyaline papilla occurs in *Lejeunea setiloba!* The only pertinent conclusion one can derive from this evidence is that lobule form, and variation, in *Rectolejeunea*, *Lejeunea* and *Stylolejeunea* (*sensu* Sim) are meaningless in attempting to distinguish genera, and that *Stylolejeunea* should never have been proposed as a new genus. Arnell has brought out the fact that "*Stylolejeunea*" *rhodesiae* Sim has caducous leaves ("leaves brittle") and states this is also the case in the genotype "*Stylolejeunea*" *duncanii* Sim. He, indeed, states "it is thus likely that these two species are forms of the same plant." Certainly, the present study clearly shows that weak and poorly developed plants of *Rectolejeunea phyllobola*, which may not develop caducous leaves at all, may have lobules wholly identical with those of *Lejeunea setiloba*. The only valid distinctions of generic significance, which I have relied on in the key to genera (Part I of this revision, p. 122, 1955) at such times wholly fail, yet I know of no others that can be used.

Lejeunea longifissa Steph.

Lejeunea longifissa Stephani, Sp. Hep. **5**: 747, 1915; Evans, Amer. Jour. Bot. **5**: 137, fig. 3, 1918.

Plants in decumbent, thin, irregular mats, somewhat to prominently *shiny when dry, pure to whitish-green, rather pellucid*, irregularly and rather sparsely branched (except for sexual branches and innovations); *shoots (650) 760–950, rarely up to 1000–1100 μ wide*; stems 50–70 μ in diam. the ventral cortical cells relatively thin-walled, (17) *19–25 μ wide* × 28–56 μ long. Leaves contiguous to loosely imbricate, the lobe *nearly flat* when dry or moist, obliquely to widely spreading (55–75°), *ovate, widest clearly in the basal fourth*, sometimes more or less obovate on juvenile leaves; mature leaves from (360) 400–510 μ long × (240–260) 300–370 μ wide, up to a maximum (to our south) of 600–670 μ long × 400–420 μ wide, gradually narrowed from the basal 0.2–0.3 to the apex, the antical margin moderately to strongly arched, the postical nearly straight or weakly convex, the apex rounded (often rather narrowly so) to very bluntly pointed or obtuse, the margins entire or slightly sinuous, usually distinctly *crenulate due to bulging cells*; arched antical margin basally extending at most to stem midline, less rarely partly across stem. *Lobules polymorphic*, on fertile stems usually somewhat reduced or vestigial (*ca.* 90 μ long × 30 μ wide or smaller, terminated by 1-celled tooth), on innovations and sterile stems usually at least in part large and inflated, rarely all vestigial; inflated lobules broadly ovate to subglobose, inflated throughout, *ca.* 120–165 μ long × (95) 100–125 μ wide; free margin involute to apical cell; apical cell obtuse, straight, blunt, with hyaline papilla at proximal base; *lobules of maximal size ca. 0.25–0.3 of the length of lobes*, slightly to distinctly smaller than underleaves (as outlined) in area; keel weakly to moderately arched, with at most a shallow and obtuse notch at juncture of its apex and the free dorsal lobe. Cells thin-walled, *pellucid*, rather obviously convex, but with distinct, usually slightly *nodulose trigones and occasional to ubiquitous intermediate thickenings; marginal cells 17–20 μ, median cells (22) 23–26* × *23–28 (33) μ*, below the middle increasing to 25–30 × 30–35 (up to 40–45) μ; *oil-bodies segmented-papillose, usually (2) 3–7 per cell*, elliptical to linear and 2.5–3 × 8–11 μ to 4 × 5 μ, a few spherical and about 4 μ; chloroplasts 3.5–4.2 μ. Underleaves *relatively small and distant*, ovate to suborbicular in outline, from 95 μ wide × 140 μ long to 150–160 μ wide × 155 μ long, up to a maximum of 175 μ wide to 185–205 μ long, *averaging as long as or longer than broad, deeply bifid* (0.6–0.85 of their length), the *sinus broadly U-shaped to lunate*; lobes erect, *divaricate or incurved, lanceolate to linear-lanceolate, usually 4* (rarely 3 or 5) *cells wide at base, often tipped by 2 superimposed cells*; lateral margins entire or *weakly unidentate* due to a protuberant cell, or irregularly sinuous; rhizoid-initials usually few, 6–10.

Autoecious. Androecia usually forming sessile lateral branches, not proliferating apically, of 2–5 (6) pairs of bracts, 360–440 μ wide; rarely androecia terminal on elongate leafy branches; bracteole at base only, similar to underleaf but smaller; 1–2-androus. Female inflorescences indiscriminately *on short lateral branches* (bearing a single leaf) *or on elongate, leafy branches*; with usually 1, rarely 2 subfloral innovations; *innovations* (of both cladogynous and acrogynous inflorescences) *usually soon again fertile*, only rarely remaining short and sterile. Female bracts extremely variable, those of cladogynous inflorescences usually much smaller, sometimes somewhat dentate; those situated on long, leafy shoots usually larger, lacking teeth. Bracts of acrogynous inflorescences with lobes to (400) 540–640 (700) μ long × (200) 340–380 μ wide, but often smaller, oblong to ovate or obovate, obtuse to subacute, with narrowly rounded or obtuse apex, or rounded distally; lobule variable, *usually lanceolate-acuminate* (at least on one side; the other lobule often lingulate with rounded, truncate or bidentate apex), often terminated by 2 superimposed cells, *large*, up to (260) 360–480 μ long × (70) 110–130 μ wide (*its length 0.65–0.9 of that of lobe*), *free for (0.35) 0.6–0.85 of its length*, the sharply complicate *keel usually short*; bracteole shortly connate or virtually free, oblong, mostly 460–490 μ long × 235–280 μ wide, *deeply bifid*, the narrow, closed sinus descending (0.35) *0.45–0.65, occasionally to 0.8 of the length*, the lobes acute to short-acu-

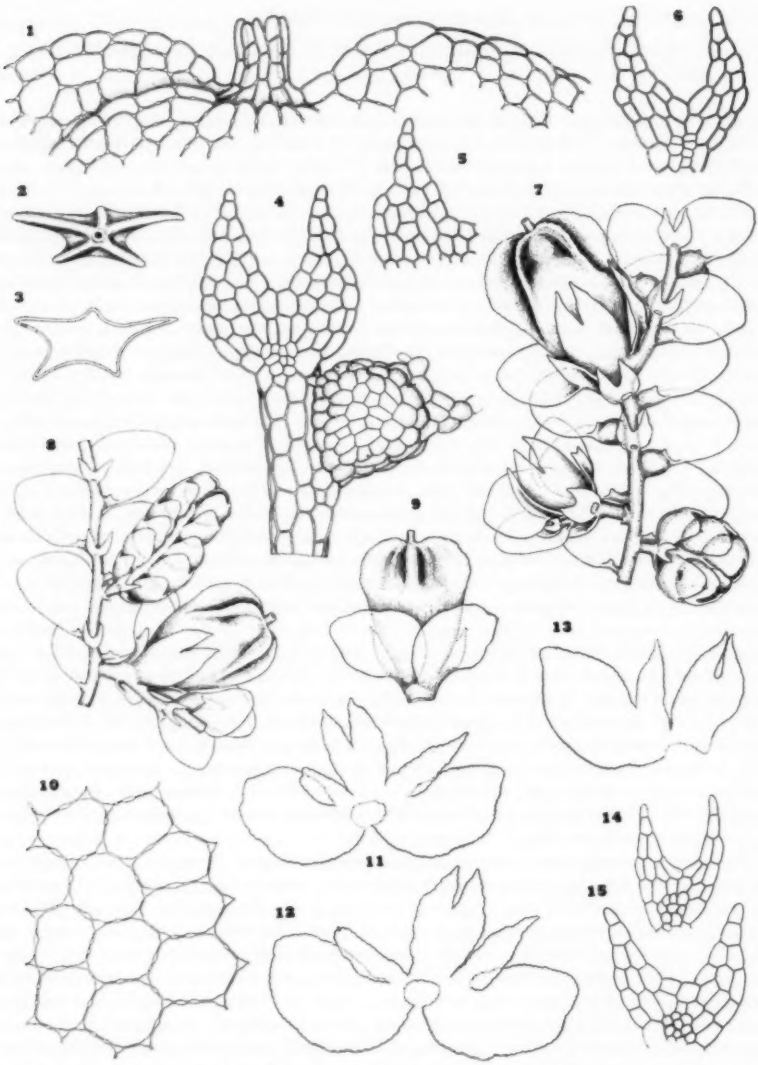


FIG. XLII. *Lejeunea longifissa* Steph. 1. Apex of perianth (×205); 2. Apex of perianth (×47); 3. Cross-section of perianth, one-third from apex (×47); 4. Lobule and underleaf, *in situ* (×135); 5. Lobe of large underleaf (×135); 6, 14–15. Underleaves (×135); 7–8. Portions of fertile plants (×38); 9. Perianth and bracts, antical aspect (×38); 10. Median cells (×300); 11–13. Bracts and bracteoles (×38). (All drawn from *Heller 147*, Bayamon, Puerto Rico; admixed with *L. minutiloba* and *L. pililoba*).

minate, often terminated by 2 superimposed cells, erect, occasionally sinuous or repand on margins; margins of both bracts and bracteole slightly to distinctly crenulate due to bulging cells. Bracts of cladogynous inflorescences often with outer bract broad and blunt, the margin entire to irregularly sinuous or coarsely toothed, the inner bract usually sharp and acute; lobule of outer bract usually blunt and often bidentate at apex; lobule of inner bract usually lanceolate and slender, more or less acuminate. Perianth large, (350) 480–530, occasionally 625 μ wide × 650–800, occasionally 850–950 (1200) μ long, *obovoid-obcordate, rather strongly dorsiventrally compressed, the antical keel obtuse and short*, the postical extending down for at least 0.6 of the perianth length; *lateral keels sharp, compressed*, dilated, often narrowly and locally winged, broadly rounded distally into the *truncate-emarginate apex*, the beak elongate, *situated usually in a distinct incision*; beak of 2–3 tiers of cells, the cells 8–10 (12) × 15–25 μ, scarcely finger-like, *never internally produced*.

TYPE: Cuba.

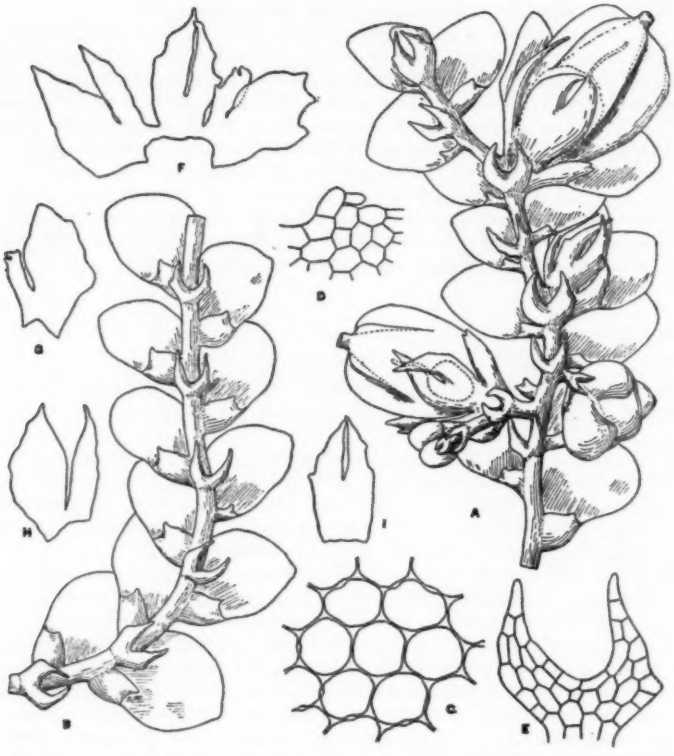
DISTRIBUTION.—Apparently a rare and local species, of West Indian origin, ranging from Puerto Rico (*Pagan, 1939;* also Bayamon, *A. A. Heller 147*! NYBG, *p.p.*, among *Lejeunea minutiloba, L. pililoba*), to Cuba (type; also Monte Verde, *Wright 1859*), and the tropical and subtropical parts of Florida.

Florida: SEMINOLE CO. (Sanford, *Rapp 83!; fide* Evans, 1918); HILLSBOROUGH CO. (Hillsborough R. in Hillsborough R. State Park, *RMS 33909a, p. min. p.*); DADE CO. (Ross Hammock on Hainlin Drive, near Silver Palm, *RMS 33801, p.p., c. caps.*; Costello Hammock, near Silver Palm, *RMS 22948a, p. min. p.*, among *Rectolejeunea phyllobola*). There are also erroneous reports from Dade and Hendry Cos. (Redfearn, 1952).

I have studied four specimens, collected and determined by Redfearn as "*L. longifissa*," and published as such (Redfearn, 1952). All of them clearly are extremes of *Rectolejeunea* with caducous leaves not developed. Three of them consist of the merest scraps and are not certainly determinable, even by the specialist, although the plant from Hendry Co. is almost surely *R. brittoniae* (marginal cells 14–15.5 μ; plants not clearly autoecious); a second collection, from Dade Co., is almost certainly also *R. brittoniae* (marginal cells 13.5–15.5 μ; no demonstrable autoecious inflorescences), while the third (Dade Co.) seems to be *R. phyllobola* (marginal cells 12.5–15 μ). The small marginal cells, hexagonal median cells, reduced lobules, and underleaf form, as well as the entire facies, reveal these plants to be *Rectolejeunea*, even at a glance. The fourth collection is more copious, clearly autoecious, and clearly belongs to *R. phyllobola*; it is from a "Low Hammock in Everglades National Park, Dade County."

ECOLOGY.—Corticolous and (rarely) epiphyllous. On bark found with other species of *Lejeunea* (in *Heller 147*, from Puerto Rico with *L. minutiloba* and *L. pililoba*, and other Lejeuneaceae). In Ross Hammock occurring in deep virgin hammock forest, at less than 20 ft.-c. light intensity (maximum?), on bark, together with *Plagiochila ludoviciana, Radula australis, Lejeunea minutiloba, L. laetevirens, Ceratolejeunea laete-fusca,* and the moss, *Neckera disticha* Hedw. Found a single time, in small quantity (*Schuster 33909a*) in the pleated, conduplicate portions of the base of the leaf-blade of the Saw Palmetto, *Serenoa repens*, in open hammock forest. There associated with *Lejeunea flava, Cololejeunea* cf. *myriocarpa, Frullania obcordata, Leptocolea cardiocarpa*, and *Diplasiolejeunea rudolphiana*.

DIFFERENTIATION.—A small to medium-sized, pure or whitish-green, pellucid plant which is usually strongly shiny. The color and flat leaves as well as the size may suggest a rather weak *L. glaucescens*; this similarity is further empha-



[FIG. XLIII. *Lejeunea longifissa* Steph. A. Part of plant, postical aspect, with three gynoecia and one androecium (×50); B. Part of sterile shoot, postical view (×50); C. Median leaf cells (×300); D. Apex of lobule, sinus at left (×225); E. Underleaf (×225); F. Bracts, and a bracteole (×50); G–I. Bracts and bracteole from one gynoecium (×50). (From Evans, 1918; all drawn from *Rapp 83*, Sanford, Florida).

sized by the strongly ovate or oblong-ovate leaves (Fig. XLII: 7–8), the relatively large median cells, and the segmented oil-bodies (Fig. XLIV: 10). The bracts of *L. glaucescens* may also be somewhat similar, although never repand-dentate, and the lobules of the bracts are often similarly acute (although rarely to as high a degree). *L. longifissa*, however, is not closely allied to *L. glaucescens* or any other regional species of *Lejeunea*. The deeply bifid underleaves and the compressed perianths strongly suggest the species of the subgenus *Stylolejeunea*.

The perianth form of *L. longifissa* is of particular interest in the light it throws upon the extremely close relationship, and evident intergradation, between *Lejeunea* and *Rectolejeunea*.

The plants from Puerto Rico here illustrated (Fig. XLII) differ in some important respects from the Floridian plants (Hillsborough Co.) studied by the writer and those (Seminole Co.) studied by Evans, whose plate of the Sanford,

Fla., material is here reproduced (Fig. XLIII). The Puerto Rican plants have the bracts with usually obovate to oblong lobes, entire-margined except for the faint crenulations (Fig. XLII: 11–13); the lobules of the bracts are also usually acute-lanceolate or even acuminate, on at least one side of the inflorescence (on the other they may be rounded-truncate or emarginate, with the hyaline papilla situated in the emargination). Perhaps even more important is the perianth form of the Puerto Rican material. The perianths in this are almost always obcordate, with the relatively slender and long beak situated in a terminal notch (Fig. XLII: 1, 7, 9). Furthermore, the perianths are quite prominently dorsiventrally compressed, with the antical keel often very low and almost vestigial (Fig. XLII: 2–3). Evans does not mention any perceptible flattening for the perianths of the Floridian plants, although he states that the dorsal keel is shorter than the two ventral. In spite of these discrepancies, there seems no doubt but that a single species is at hand. (The plants from Dade Co., Florida, completely agree with the Puerto Rican plants in the form of the perianth.)

A final peculiarity is noteworthy. The leaf cells have commonly distinct, if rather small, almost nodulose trigones, and show usually a free development of intermediate thickenings (Fig. XLII: 10). This is even more highly marked on the female bracts, and most accentuated on the cells of the perianth, where the trigones and intermediate thickenings may be prominently nodulose (Fig. XLII: 1). The extreme shade forms (*Schuster 33801*, Dade Co., Florida) show this feature to a lesser extent, although even here the older leaves, bracts and perianths are quite collenchymatous in some cases (Fig. XLIV: 14).

The chief diagnostic characters of the species are: (a) the deeply bifid underleaves, usually with a broad, sometimes lunate sinus, with the lobes narrow, 2–4 cells wide at base, and 2–4 times as long as wide; on one or both sides of some underleaves there may be an obtuse tooth or angulation, much as in *Lejeunea laetevirens* (Figs. XLII: 4–8; XLIII: B, E; XLIV: 1–2, 4, 9); this last feature does not recur in any other of our *Lejeunea* species; (b) the polymorphic or often dimorphic lobules, which are mostly vestigial on fertile shoots, but inflated and ovate and well-developed on many leaves on sterile (less frequently fertile) shoots (Fig. XLII: 4, 7–8); similar polymorphic lobules recur again in the related *L. cladogyna*, *L. minutiloba* and *L. glaucescens*; (c) the perianth has the lateral keels somewhat more strongly expanded than the ventral, while the dorsal is lower and weaker than the other keels; consequently, the perianth is compressed, with the lateral keels often vaguely winged locally. The lateral keels are so strongly developed, particularly distally, that the perianth is, in dorsal profile, rounded-truncate at the apex, or often even slightly retuse at the insertion of the beak (Fig. XLII: 1, 7–9). In the compressed perianth, with low dorsal keel, and prominent lateral keels, *L. longifissa* closely approaches *Rectolejeunea*; (d) the female bracts are nearly free from the bracteole, and the bracts bear lobules that are often narrowly lanceolate and acuminate or acute (Fig. XLII: 11–13); these lobules may be sometimes nearly as long as the rounded to acute or often obscurely coarsely dentate or dentate-angulate dorsal lobes; the lobules are usually united with the lobes by only a short keel. The bracts are usually one-half or more the length of the perianth.

The form of the underleaves, which often bear an obtuse to lunate sinus and have lobes mostly only 3–4, rarely 2 or 5, cells wide at base, suggests some similarity to *L. cladogyna* and *Rectolejeunea phyllobola*, particularly to the latter. The polymorphic lobules, which are usually obsolete on fertile plants, further emphasize this similarity—as does the small size of the underleaves, which are at most twice the width of the stem. The truncate to truncate-emarginate perianth, and the relatively large bracts and bracteole, frequently lunate underleaf-sinus, suggest that *L. longifissa* is related to *Rectolejeunea phyllobola*. The latter differs from *L. longifissa* in the less acuminate lobes of the underleaves, which may be as much as 5–6 cells wide at base; the wider underleaves (which often average somewhat wider than long); the usual lack of a tooth on one or both external margins of the underleaves; the much shorter lobule of the bracts, usually less than 0.6 as long as the dorsal lobe of the bracts; the bracts are also never dentate, and the lobule never acuminate. The two taxa are surely separable, even when wholly sterile, on the basis of the leaf cells; those of *L. longifissa* are larger (17–20 μ) on the margins, and the median are thin-walled but distinctly collenchymatous and provided with intermediate thickenings. *Rectolejeunea phyllobola*, by contrast, has marginal cells 12–17 μ (averaging usually 13–15 μ), and the cells are not thin-walled with nodulose trigones and intermediate thickenings. An additional distinction which is very obvious when the two species occur admixed is the shiny texture of *L. longifissa*, and the pellucid appearance, as contrasted to the dull texture and opaque appearance of *R. phyllobola* and the other species of *Rectolejeunea*.

The sparse material seen from Hillsborough Co., Florida, differs from the Puerto Rican material in several noteworthy respects, chiefly in (a) the strong tendency towards formation of obovate leaves, constricted in their basal 0.25–0.35, on weak shoots (Fig. XLIV: 6–8); (b) shinier texture, when dry; (c) much smaller, although discrete, trigones and sporadically distributed intermediate thickenings. In other respects, however, these plants are quite typical, particularly in cell-size and form of the underleaves (Fig. XLIV: 1–11). In their deviant features, these plants very closely approach *L. cladogyna*, with which they agree in the segmented oil-bodies and tendency towards development of narrow-based, obovate leaves. However, even these plants are clearly distinct from *L. cladogyna* in their larger cell size and in the extraordinarily attentuated lobes of the underleaves.

The fertile and abundant plants from Ross Hammock, Dade Co. (*Schuster 33801*), are also quite deviant in some respects, such as (a) smaller size and more distant leaves, the shoots rarely over 650 μ wide; (b) the narrow and pronouncedly ovate leaves (Fig. XLIV: 15), the larger only 360–400 μ long $\times$ 240–280 μ wide (1.4–1.5 times as long as wide); (c) leaves uniformly with vestigial lobules (Fig. XLIV: 13, 15); (d) bracts with lobes smaller than described, their lobes more or less obovate, always broadly rounded distally; the lobules often free for only 0.3–0.4 of their length, often less than 0.8 of the length of lobes; (e) trigones and intermediate thickenings often weakly developed. These plants appear to be an extreme shade form (mod. *angustifolia-elobulata*). In their strongly nitid appear-

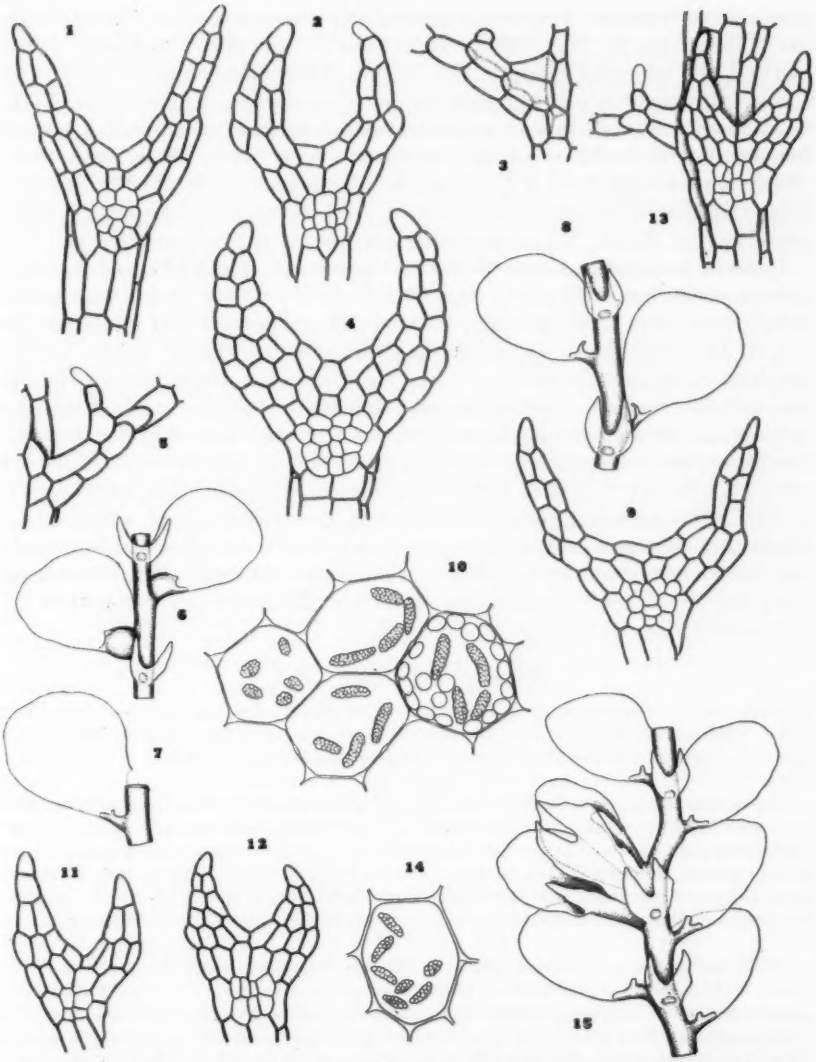


FIG. XLIV. *Lejeunea longifissa* Steph. 1–2, 4, 9. Underleaves of mature form (×212); 3, 5. Reduced lobules (×212); 6. Shoot-sector, postical view, with inflated lobules (×48); 7–8. Shoot-sectors, postical view, with vestigial lobules (×48); 10. Median cells (×650); 11–12. Small underleaves (×205); 13. Shoot-sector with underleaf and lobule (×205); 14. Median cell (×665); 15. Shoot-sector, postical view, with gynoecium (×60). (Figs. 1–11, *Schuster 33909a*, Florida; 12–15, *Schuster 33801*, Florida).

ance, and the segmented oil-bodies, they closely matched the "sun" plants from the Hillsborough R., but differed from them in the almost uniformly ovate leaves.[35] The underleaf shape, in this species, unfortunately appears subject to considerable modification. Although the underleaves on most plants of the Dade Co. material were quite typical, with attentuate (occasionally connivent) lobes and frequent lateral teeth, occasional underleaves were barely longer than wide, closely approaching those of *L. cladogyna* in appearance (Fig. XLIV: 12). The great variation in position of the gynoecia, with the majority on elongate, leafy branches (Fig. XLIV: 15), at once eliminates the latter from consideration.

It should be noted that both the Puerto Rican plants (Fig. XLII) and the more slender, somewhat laxer plants from Florida (*RMS 33801*) have bracts which differ greatly from those figured by Evans (1918; his figure is here reproduced as Fig. XLIII). The plants studied by the writer show a virtually uniform lack of dentition of the lobes of the bracts, and their apices are almost always broadly rounded. Furthermore, their lobules may be less than half the length of the lobe (Fig. XLII: 8) and are sometimes free for less than half their length. The bracteoles, also, are much less distinctive than those figured by Evans. They are often bifid for only 0.35–0.45 of their length, with otherwise entire margins (but in *RMS 33801* not rarely provided with a small lateral tooth on one or both sides). Study of the limited available materials suggests that *L. longifissa* is an extremely polymorphous taxon, best delimited by the unique underleaves, the rather large cells, the ovate leaves, segmented oil-bodies, the frequently acrogynous inflorescences, and the shiny texture when dry.

Lejeunea minutiloba Evs.[36]

Lejeunea glaucescens Evans, Bull. Torrey Bot. Club **33:** 131, 1906 (in part; nec Gottsche); Schuster & Hattori, Jour. Hattori Bot. Lab. No. 11: 51, pl. XI: 4–5, 1954.[37]
Lejeunea minutiloba Evans, Bull. Torrey Bot. Club **44:** 525, pl. 24, 1917.

Plants pure, clear green, in drying or with age often brownish, *strongly glistening when dry, pellucid*, in depressed patches or mats, or scattered; *shoots delicate, mostly 640–780 (960–1000) μ wide*, freely and rather irregularly 1–2-pinnate, the branches obliquely to widely spreading. Stems 70–75 μ in diam.; ventral cortical cells *ca.* 26–30 μ wide × 48–58 μ long. Leaves approximate to weakly imbricate, on weak shoots often with distant, *smaller, obovate leaves* (but not microphyllous), obliquely to widely spreading (60–75°), nearly flat.

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[35] The rather sparse and sterile plants of *Schuster 22948* also represent an extreme shade phase with trigones and intermediate thickenings poorly developed. These plants, however, possess distinctly dimorphic lobules, with a minority large and inflated, most obsolete. The underleaves in these plants are quite typical, with their horn-like, slender, drawn-out lobes and lunate sinus; therefore the material cannot be placed elsewhere! Particularly notable in this material is the very copious development of rhizoids, which issue from a distinct, almost circular disk, as many as 15–24 rhizoids occurring at each underleaf base.

[36] The similarity in names should be noted between the present species and the Japanese *L. minutilobula* Horikawa, as well as the African *L. microlobulata* S. Arn.

[37] The collection figured and described in Schuster and Hattori (1954) is a mixed collection, with both *L. glaucescens* and *L. minutiloba* present. The dull plants are *L. glaucescens*; the shiny plants, from which the cytological data were taken, are *L. minutiloba* var. *heterogyna*.

Dorsal lobe flat, *becoming somewhat concave when dry*, when well-developed *broadly ovate to suborbicular* (widest somewhat above the base), varying from 330–350 μ wide × 375–400 μ long, up to 450 μ wide × 500 μ long; *dorsal margin arching only partly* (rarely wholly) *across stem*, then strongly outwardly arched to the *broadly rounded apex*; posterior margin straight to slightly convexly curved, the margin entire to obsoletely crenulate (due to barely projecting marginal cells); *dorsal lobes of ultimate branches, and of weak shoots, often obovate*, widest above middle and strongly narrowed towards base, the apex very broadly rounded. Lobules *uniformly reduced to minute basal folds*, formed of fewer than six cells, the apex marked by a single projecting cell, whose apex is tipped by the clavate hyaline papilla. Cells relatively small: *marginal cells 13–16 (17–19) μ; median cells (18) 20–23 (27) × 25–27 (30–35) μ* (in isolated leaves occasionally up to 23–27 × 28–32 μ); cells more or less leptodermous, with minute to moderate trigones and few or no (rarely frequent) intermediate thickenings; *oil-bodies 8–15, 16–20, or even 30–65 per cell, homogeneous* (or faintly transversely barred with age), *small*, 2–3 μ to 1.8–2 × 3–5 μ, a few up to 2 × 6–7.5 μ, *glistening*, averaging smaller or subequal to the chloroplasts in size. Underleaves variable: *distant* (separated by a distance approaching or exceeding their length), *suborbicular* to *orbicular*, occasionally slightly wider than long, varying from 145 × 145 μ to 145 μ long × 185 μ wide, occasionally up to 200–205 μ long and wide, *averaging twice as wide as stem*; sinus descending through 0.5–0.6 of the length, V-shaped, with base acute to narrowly rounded; lobes erect to barely divergent, triangular, *5–6 (7–8) cells wide at base*, the margins entire or faintly crenulate due to bulging cells, the base rounded to cuneately narrowed, never cordate; underleaf cells *ca.* 14–18 × 16–23 μ.

Autoecious. Androecia almost always on short lateral branches, without vegetative leaves, usually not proliferating at apex; bracts in 2–4 pairs, closely imbricate, the androecium shortly spicate, *ca.* 320 μ wide; bracts strongly inflated, shortly and subequally bifid, with keel strongly arched, crenulate with bulging cells; lobes rounded; bracteoles limited to bases of androecia, like underleaves but smaller. *Female inflorescences variable in position: often at apices of leading, leafy branches*, sometimes on a more or less abbreviated lateral branch; innovation one, *often again floriferous*. Bracts suberect to obliquely spreading, varying greatly in size (depending on position of inflorescence). Bracts of terminal inflorescences large, the oblong to obovate lobe 350–400 (450) μ long × 205–230 (300) μ wide, entire-margined; *lobules small*, narrowly lingulate, usually *rounded at apex*, free for ⅓–⅕ of their length, 160–180 μ long × 40–60 μ wide; bracteole 360–400 μ long × 250 μ wide, obovate usually, bilobed *ca.* ⅓, the erect lobes acute, obtuse, or sometimes rounded. Bracts of inflorescences of lateral branches much smaller: the lobe 250–340 μ long × 125–200 μ wide; the lobule 80–160 μ long × 30–40 μ wide; bracteole 280–350 μ long × 180–215 μ wide. Perianth obovoid, when well-developed to 350 μ wide × 500 μ long (but perianths of cladogynous inflorescences often only 250 μ × 400 μ), terete in basal half, the *distal third* sharply 5-carinate, the keels distally suddenly, abruptly constricted to the beak, the perianth thus *truncate distally or even with a slightly recessed beak*; perianths usually exserted for 0.35–0.45 of their length, on cladogynous inflorescences sometimes up to 0.5–0.6 of their length.

TYPE: Crown, St. Thomas, Virgin Isls., W. I. (*Britton and Marble 1365*); NYBG, Y.

DISTRIBUTION.—A tropical and subtropical species, ranging from the West Indies (Crown and St. Peter, St. Thomas, Virgin Isls.; Rio Piedras, Bayamon, Coamo Springs, Ponce, Candalaria, and between Lares and San Sebastian, Puerto Rico; San Luis, Oriente, and Puentas Grandes, Havana, Cuba) to Bermuda (Walsingham, Church Cave, Tuckerstown, Abbott's Cliff), and northward to the extreme Coastal Plain of the southeastern United States:

Florida: DADE CO. (Mathesson Hammock, near Coral Gables, *RMS 22632, p.p.*, the shiny plants only, the dull ones being *L. glaucescens*; Paradise Key, Everglades Ntl. Park, *RMS 19901 and 22784, p.p.*, among *Rectolejeunea phyllobola*; Paradise Key, *RMS 20161, p.p.*,

among *R. brittoniae* and *Caudalejeunea*; on leaves of *Tectaria heracleifolia*, Timms Hammock, Bauer Drive, *RMS 33701g and 33701h, p.p.*; bark and twigs of *Pisonia aculeata*, Timms Hammock, *RMS 22856a*); COLLIER CO. (Cypress Head near Immokalee, *M. S. Taylor 1700!*); CITRUS CO. (Over coral limestone, Grottos at Pineola, *RMS 37213a, 37224b, 37228, 37231a, p.p.*, with *Dumortiera hirsuta, Cololejeunea contractiloba, C. ornata* and *Plagiochila ludoviciana*); DE SOTO CO. (Roots, Bay Hammock 5 mi. n. of Arcadia, *Redfearn, July 1949*); ALACHUA CO. ("Buzzards Roost," *ca.* 7 mi. w. of Gainesville, *RMS 33388, 33349, 33410, 33359, p.p.*). Also reported from Polk Co. by Redfearn (1952). **Louisiana:** Avoca I., near Morgan City (*Svihla 400, teste* Frye & Clark, 1947).

I have also seen a few plants, growing over the filmy fern, *Trichomanis petersi*, from Mt. Olive, Simpson Co., Mississippi, in which the cells had small, homogeneous oil-bodies. These plants almost surely belong to *L. minutiloba*, but the material is too sparse and sterile for certain identification.

The plant from De Soto Co., Florida, cited and determined by Redfearn (1952) as *L. cladogyna*, is rather frequently acrogynous and has bracts with small lobules; therefore is *L. minutiloba*. The plants are transitional to the var. *heterogyna* of *L. minutiloba* in (a) the usually small *L. cladogyna*-like underleaves; (b) a single leaf was found with an inflated lobule—otherwise all of the leaves had rudimentary lobules. The inflorescences were frequently on elongate, leafy, lateral branches and had, then, innovations which were indeterminate in length. I have examined the voucher specimen (University of Tennessee Herbarium) from Polk Co., Florida, cited by Redfearn (1952); it is a sterile and wholly undeterminable scrap; therefore the Polk Co. report cannot be accepted. I have not seen the Louisiana collection cited by Frye and Clark (1947), and doubt the occurrence of the species in Louisiana. Indeed, the Svihla collection, first reported by Svihla (1939), and the Redfearn collection are both probably to be referred to other species.

ECOLOGY.—*L. minutiloba* locally is generally associated with outcrops of coral limestone. It is frequent in rather shaded, but hardly damp sites on the coral limestone walls of "grottos" (as at Buzzards Roost near Gainesville, and at Pineola), but also occurs around limesinks in the southern tip of Florida. Around small and deep limesinks it is not restricted to limestone, but may be corticolous or rarely even epiphyllous. For example, at Timms Hammock it is found on the leaves of the large halberd fern, *Tectaria heracleifolia*, associated with *Lejeunea flava, L. minutiloba heterogyna, Aphanolejeunea cornutissima* (*A. evansii*), *Caudalejeunea lehmanniana, Cololejeunea subcristata* and *minutissima myriocarpa, Rectolejeunea phyllobola* and *Lopholejeunea subfusca* (*RMS 33701a-h*). More typical than the occurrence on leaves is that on bark of lianas, as of *Pisonia aculeata* (with *Caudalejeunea* and *Rectolejeunea phyllobola*), or further northward on lianas of *Vitis* (with *Crossotolejeunea bermudiana, Cololejeunea contractiloba*). In the tropical hammocks of southern Florida also occasionally on dead twigs and branches on the damp hammock floor, then often with *Lejeunea glaucescens*. To our south (Puerto Rico) often on tree trunks, and then with *Lejeunea longifissa* and *L. pililoba*. In the hammocks of southernmost Florida also frequent on the bark of trees, usually in deep shade, and often only as scattered, shiny individual plants, associated with, most often, *Rectolejeunea brittoniae* and *phyl-*

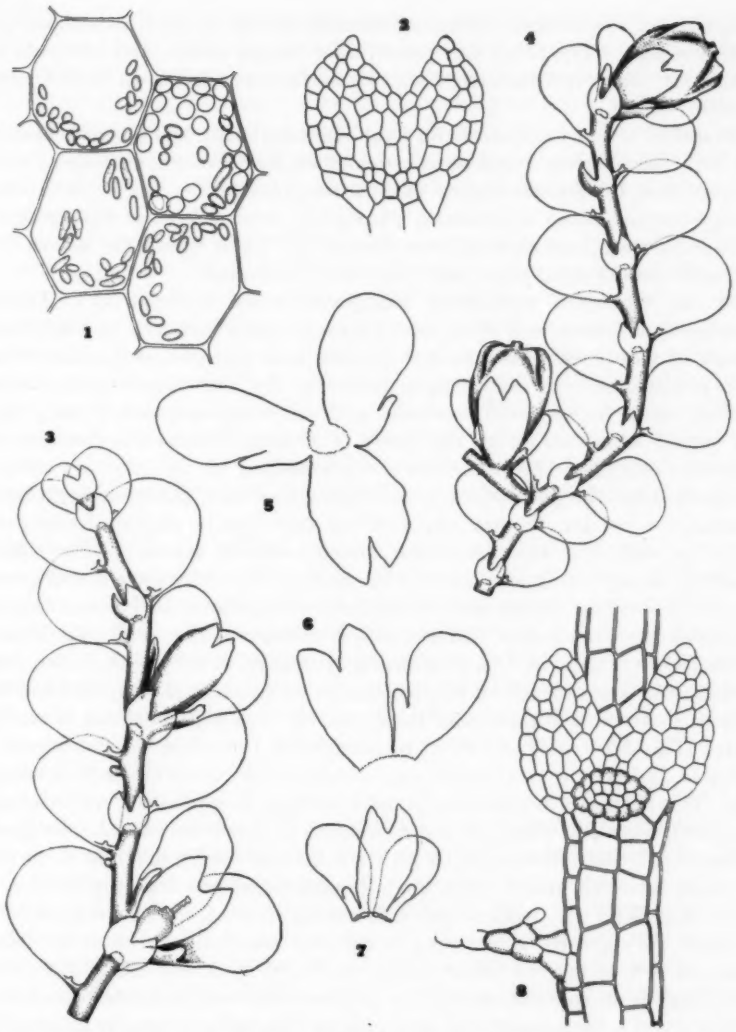


FIG. XLV. *Lejeunea minutiloba* Evs. 1. Median cells with oil-bodies, in one cell also with chloroplasts drawn in (×768); 2. Underleaf (×137); 3. Shoot with acrogynous gynoecium (×42); 4. Shoot with acrogynous and cladogynous gynoecia (×42); 5. Bracts and bracteole from acrogynous gynoecium (×57); 6–7. Bracts and bracteoles from cladogynous gynoecia (×57); 8. Underleaf and lobule, *in situ* (×155). (Figs. 1–3, *Schuster 33359*. Florida; 4–7, *Schuster 22856a*, Florida; 8, *M. S. Taylor 1700*, Immokalee, Florida).

lobola, *Caudalejeunea lehmanniana*, occasionally *Mastigolejeunea auriculata*. Such corticolous phases are, in my experience, often largely sterile, and represent the most robust, nearly unbranched extreme of the species, ranging up to 950–1000 μ in width.

The species is found frequently on coral limestone ledges and the walls of sinks, then with various ferns (*Asplenium verecundum*, *heterochroum*, *abscissum*, *cristatum*, *pumilum*, *Goniopteris reptans*, etc.), as near Gainesville, Fla.; it there occurs among *Crossotolejeunea bermudiana*, *Plagiochila dubia*, *Reboulia hemisphaerica*, *Lejeunea calcicola*, *Dumortiera hirsuta*. Evans (1917) also reports the species from bark, and roots of royal palm, and from stones and earth.

The var. *heterogyna*, apparently widespread in the southern tip of Florida, occurs there as a hammock plant, over twigs and small branches, and sometimes on bark of tree trunks, associated frequently with *Lejeunea glaucescens* (which differs in the larger size, duller appearance when dry, and ovate leaves), and occasionally with *Rectolejeunea brittoniae*, and other Lejeuneaceae. It may occur with typical *L. minutiloba* on the leaves of *Tectaria heracleifolia* (see above).

DIFFERENTIATION.—*L. minutiloba* is characterized by the relatively deep or pure green color, the great delicacy, and rather small size, the strongly glistening appearance when dry, the flat leaves (which may even be slightly dorsally concave in drying), and the suborbicular, broadly distally rounded, often slightly distant or merely contiguous leaves (Fig. XLV: 3–4), which have a strong tendency (on less robust shoots and on subfloral innovations) to become obovate. The plants agree in all these features with *L. cladogyna*. The small cells (those of the margins averaging 15–18 μ or occasionally smaller, at the leaf-tips); the strong tendency for development of obovate leaves on juvenile shoots, that are conspicuously narrowed in their basal third; and the very high incidence of vestigial lobules (Fig. XLV: 3–4, 8) serve to accentuate this close relationship to *L. cladogyna*. Indeed, the two species may be superficially quite identical in size and facies. This similarity is especially marked between *L. minutiloba* var. *heterogyna* (described below), in which occasional inflated lobules occur, and *L. cladogyna*.

However, *L. minutiloba* agrees with the otherwise totally different *L. cavifolia* in having relatively many, very small, homogeneous and highly glistening oil-bodies (Fig. XLV: 1), while those of *L. cladogyna* are few and compound. The oil-bodies vary greatly in number, in different populations, but range from a minimum of 8–15 per cell (*RMS 33359*) to (9–16) 16–20 per cell (*RMS 37224*). They range from spherical and 2–3 μ to more often ellipsoidal to fusiform and 1.4–2.4 × 3–6 μ. Occasionally leaves are found with rather unusually large median cells (22–25 up to 27 μ wide × 30–40 μ long!), which then bear up to 30–60 oil-bodies each, forming a nearly opaque mass, with the consequence that the cells are nearly opaque (*RMS 37228*). In addition to the distinctive oil-bodies, which allow a ready recognition of the species, *L. minutiloba* also differs from the superficially similar *L. cladogyna* (as well as almost all other *Lejeunea* species of our area) in the uniformly obsolete lobules. Other differential features, from *L. cladogyna*, are: (a) the occurrence of the female inflorescence commonly on main, leafy branches, more occasionally on short lateral branches (Fig. XLV: 3–4);

(b) the small lobules of the female bracts, usually rounded at the apex (Fig. XLV: 5–7); (c) the lack of fusion between bracts and bracteole; (d) the perianth carinate in only the distal ⅓, with the apex truncate or even truncate-emarginate (Fig. XLV: 4). In spite of the number of these differences, it is still questionable as to how sharp and constant some of these distinctions are. The variety *heterogyna*, described below, at first assigned to *L. cladogyna*, and now assigned with some misgivings to *L. minutiloba*, demonstrates how exceedingly tenuous these distinctions may become.

When dry material in which the oil-bodies have disappeared is studied, identification is often more difficult. Characteristics which give the plant a distinctive facies are the juvenile obovate-orbicular leaves; the suborbicular mature leaves; the vestigial lobules; the relatively small marginal cells, on normal leaves averaging only 13–17 μ; the distant, relatively small underleaves, which average as long as wide. The underleaf size and shape, and the reduced lobules of the bracts suggest forms of *L. glaucescens*. However, *L. minutiloba* differs from the latter in the homogeneous oil-bodies; lack of occasional inflated lobules; the rounded lobules of the bracts; the considerably smaller median and marginal leaf cells; shiny texture when dry; the subrotund and broad leaves; and the much smaller size.

Evans (1917), in his original diagnosis of *L. minutiloba*, compared it to *L. floridana* and *L. glaucescens*. The much smaller cortical stem cells; the form and size of the underleaves; the uniformly elobulate leaves; the perianth form; and the smaller cell-size at once separate *L. minutiloba* from *L. floridana*. The much larger, duller, *L. glaucescens* differs (in addition to the features mentioned above) in the high incidence of lobulate leaves; the constantly ovate to dimidiate-ovate leaves; the uniformly or almost uniformly cladogynous perianths. Both of these species are only superficially allied to *L. minutiloba*, as is evident from their segmented oil-bodies.

VARIATION.—*L. minutiloba*, typical, has uniformly vestigial lobules (Fig. XLV: 3–4, 8) and is characterized by relatively well-developed underleaves, whose lobes are usually 5–6 cells wide at base, or occasionally even broader (Fig. XLV: 2, 8). Both of these characteristics, of course, allow its separation from the very similar *L. cladogyna*. However, in the southern tip of Florida a plant occurs which has occasional inflated lobules (Fig. XLVI: 9; XLVII: 1, 6), and relatively small underleaves (Fig. XLVI: 1, 8–9; XLVII: 1, 8–9); this was, at first, placed under *L. cladogyna* (with which it agrees in these respects). It is here separated as follows:

Lejeunea minutiloba var. **heterogyna** var. n.

Aspectu L. cladogynae similis lobulis dimorphis interdum valde inflatis; amphigastriis parvioribus.

Mature shoots 700–840 μ wide; stems 50–60 μ in diam. Leaves up to 470 μ long × 370 μ wide, usually with vestigial lobules, but *occasionally with larger lobules* (exceptionally inflated and up to 120 μ long × 95 μ wide). Underleaves *small*, up to twice as wide as stem (from 105 μ long × 116 μ wide to 135 μ long and wide), *their lobes usually 4 cells wide at base*. Androecia up to 280 μ wide,

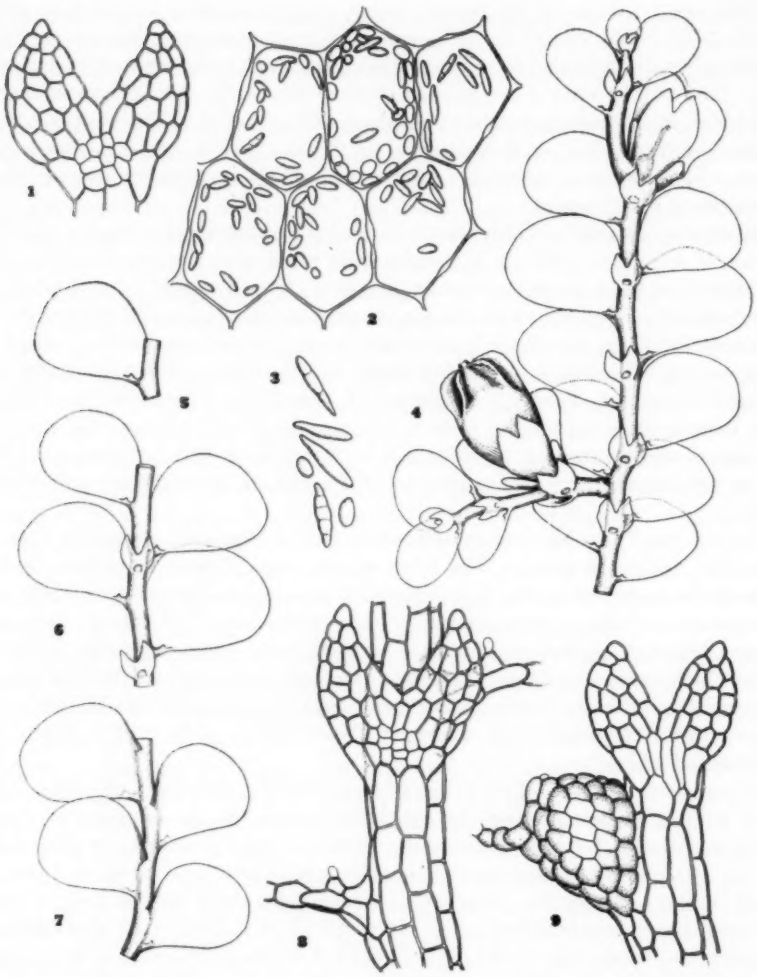


FIG. XLVI. *Lejeunea minutiloba* var. *heterogyna* Schust. 1. Underleaf (×200); 2. Median cells with oil-bodies (×645); 3. Individual oil-bodies (×1425); 4. Fertile plant with cladogynous and acrogynous inflorescences (×44); 5. Obovate leaf of sterile shoot (×44); 6–7. Sterile shoot-sectors, postical and antical views (×44); 8–9. Underleaves and lobules, *in situ*, showing dimorphic lobules (×200). (All drawn from *Schuster 22023*, paratype).

usually of 3–4 pairs of bracts, only on short lateral branches of determinate length. Female inflorescences *on leading leafy branches, or,* perhaps more frequently, *on short lateral branches.* Female bracts of inflorescences on leading branches large: up to 400–410 μ long × 180–200 (250) μ wide, *with lobules uniformly reduced in size,* 170–180 (210) μ long × 40–50 μ wide, free for *ca.* ⅓ of their length from lobes. *Bracteoles free or essentially so,* 290–340 μ long ×

200–215 μ wide, ⅓–¼ bilobed. Perianths 460–540 μ long × 320–340 μ wide, 5-carinate for ⅓ (*rarely ⅖*) *of their length*, the keels somewhat dilated above, suddenly narrowed, the perianth thus *subtruncate at apex, often with the beak slightly recessed.*

Similar to *L. cladogyna*, but (a) the female inflorescences variable in position, sometimes cladogynous, at other times on more or less elongate, leafy branches; the subfloral innovations often soon again floriferous; when inflorescence terminal on leading leafy branch, occasionally with 2 subfloral innovations; (b) bracts and bracteole hardly connate at base; (c) bracts with lobules somewhat reduced in size, varying from 0.35 to 0.50 (0.65) of the length of the lobes; (d) leaves with lobules obsolete in the vast majority of cases, inflated lobules exceptional in occurrence; (e) perianths often subtruncate at apex, keeled only for distal ¼–⅓.

TYPE: Hammock on Loveland Road, 5–6 mi. w. of Florida City, Dade Co., Florida (*Schuster 22702, 1951*); station now destroyed.

DISTRIBUTION.—Known only from central and southern Florida; evidently much rarer than the species itself, into which it must surely grade.

Florida: DADE CO. (Mathesson Hammock, Old Cutler Rd., *RMS 22686*; Timms Hammock, Bauer Drive, *RMS 22857a*; Hammock on Loveland Rd., w. of Florida City and e. of National Park line, *RMS 22702, type*; Hammock in Section 24, Long Key Pineland, Everglade Keys, Everglade Ntl. Park, *RMS 22023*; Timms Hammock, a trace on leaves of *Tectaria heracleifolia, RMS 33701f., p.p.*, in part with typical *L. minutiloba*).

The material cited under the species, from De Soto County, is transitional to var. *heterogyna*.

The variety *heterogyna* has more polymorphic bracts than *L. cladogyna*. The bracts of cladogynous perianths have the lobes sometimes only 300 μ long × 180 μ wide, with the lobule 225 μ long; at other times they are 380 μ long × 195 μ wide, with the lobule 235 μ long. On perianths from long leafy shoots, the bracts may be no larger: the lobes vary from 330–345 (410) μ long × 160–185 (200–250) μ wide, but the lobules may be much smaller (only 170–180, occasionally up to 200–210 μ long × 40–60 μ wide). The bracteoles, which are almost free from the bracts, are often smaller, 220–300, rarely 320–340 μ long × 150–190 (200–215) μ wide; they often have the sinus shallow and broad, with the lobes divergent, at other times narrow, with the lobes erect.

The androecia appear to be uniformly situated on short lateral branches; they may have 3–4 pairs of bracts, however (Fig. XLVII: 7).

In the variability (as regards position) of the female inflorescences (Fig. XLVI: 4; XLVII: 6–7); in the tendency for the female bracts to show reduced lobules about half the length of the lobes (Fig. XLVII: 2–7); in the tendency for the lobules of the bracts to be free for ⅓–½ their length; and in the more pronounced level of obsolescence of the lobules of the leaves, the var. *heterogyna* closely approaches *L. minutiloba* Evs. It also is identical with this, as regards cell-size, and form and number of the oil-bodies (Fig. XLVI: 2). In spite of the disturbingly close resemblances of this variant to *L. cladogyna*, the form of the oil-bodies definitely excludes it from that species. When only dead plants (without oil-bodies) are available, the separation of var. *heterogyna* from *L. cladogyna* becomes exceedingly difficult, and is reduced to: (a) the frequent or occasional

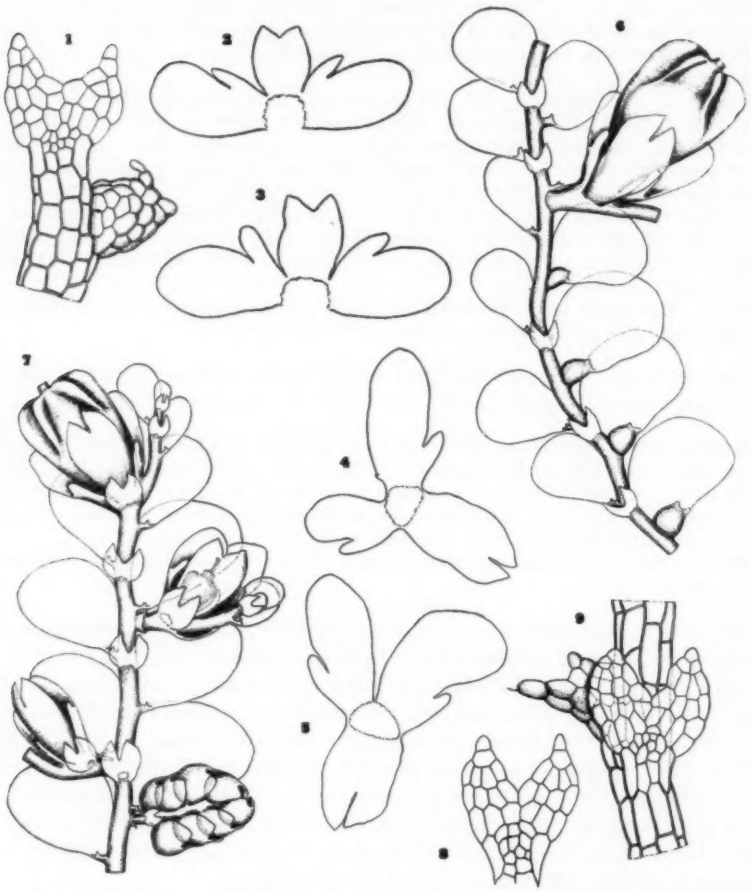


FIG. XLVII. *Lejeunea minutiloba* var. *heterogyna* Schust. 1. Underleaf and medium-sized lobule, *in situ* (×160); 2–5. Bracts and bracteoles (×50); 6. Shoot, with dimorphic lobules and cladogynous perianth (×46); 7. Shoot, with obsolete lobules, and with cladogynous and acrogynous inflorescences (×46); 8. Underleaf (×160); 9. Underleaf and small, but hardly obsolete, lobule (×160). (All drawn from *Schuster 22702*, Dade Co., Florida, type).

occurrence of female inflorescences on elongate leafy branches (Fig. XLVI: 4; XLVII: 7); (b) the ability of the subfloral innovation to grow indefinitely, and at least occasionally to develop further "inflorescences"; (c) the very strongly unequally bilobed female bracts, with the lobule often less than half the lobe in length (Fig. XLVII: 2–5); and the lack of any considerable fusion between bracts and bracteole; (d) the less sharply and longly keeled perianth, whose beak is shorter and less prominent (Fig. XLVI: 4, XLVII: 6–7).

Lejeunea cladogyna Evs.

Lejeunea cladogyna Evans, Amer. Jour. Bot. **5**: 134, fig. 2, 1918.

Pale or clear green (sometimes yellowish to brownish with age), *strongly shiny when dry*, in loose, decumbent mats. Shoots *small and delicate*, sparingly to freely, irregularly branched, on *main stems 550–800 (900) μ wide*; stems 50–75 (100) μ in diam.; *ventral cortical cells only 19–25 (28) μ wide* × 23–32 (44–48) μ long; branches of first and second orders usually with less robust stems, smaller and more distant leaves (shoots 500–800 μ wide). Leaves slightly distant to weakly imbricate, plane or slightly convex, the lobe obliquely to widely spreading (45–70°). Dorsal lobe varying from *broadly rotund-ovate* (on main axes) to, more *commonly, obovate to broadly obovate* (on main axes; most branches), *clearly if slightly longer than broad*, up to (340) 450–540 μ long × (230) 400–420 μ wide; *lobes appearing strongly narrowed at base*, dorsal margin sometimes arching slightly across axis but normally not, usually with basal portion of margin erect or suberect, then gradually, strongly, outwardly arched to the *broadly rounded apex*; ventral margin straight or slightly convexly arched. *Lobule dimorphic*: when well-developed forming a small inflated broadly ovoid sac, 90–125 μ long × 80–95 μ wide, with a straight or weakly convex keel, which is *almost continuous with free posterior border of lobe*; free margin of lobule involute to beyond the apex, the very shallow sinus oblique; apical tooth rounded, obtuse, formed by a single barely projecting cell; the *majority of lobules* (and sometimes almost all on fertile plants) *reduced to minute basal folds*, formed of only 3–8 cells. *Cells 14–17 μ on leaf-margins*, 18–23 × 23–28 μ medially (but on robust leaves of main stems sometimes locally to 20–23 μ marginally, 23–25 × 28–30 μ medially), thin-walled and with indistinct or minute trigones, with no or faint intermediate thickenings; oil-bodies few per cell, *usually 7–8 or fewer, compound, ca.* 2.5–3.5 × 5–7 μ, rarely smaller, *formed of distinct, fine, globular segments. Underleaves distant* (the intervals between underleaves 1–3 times as long as the underleaves), *small, clearly longer than broad, their width 1.0–1.7 times the stem width*, ovate to ovate-orbicular, (75) 120–140 (155) μ wide × (80) 150–180 μ long, bilobed to middle with an acute to often *U-shaped or narrowly lunate sinus, the lobes (3) 4 (5) cells wide*, erect, triangular, *blunt* to subacute, almost always terminated by single terminal cells; rhizoid-initial disk relatively small, of 5–16 small cells. Without asexual reproduction.

Autoecious. Androecia uniformly *on short, leafless, lateral branches, small* and compactly spicate, 240–300 (380) μ wide, subrotund in outline, *of only 2–3 pairs of bracts*, determinate in length (the apex not proliferating); bracts ventricose, strongly imbricate, with a strongly arched, crenulate keel and short, blunt, subequal lobes. Female inflorescences *normally on a very short, lateral branch*, bearing a single, vegetative leaf and underleaf, always with a single innovation (*normally short and sterile*); rarely with isolated gynoecia on elongated, leafy branches. *Bracts and bracteole normally connate*, at least for a short distance, *short*; bracts suberect, somewhat sheathing, the lobe oblong to narrowly obovate, from 190–270 (285) μ long × 100–125 μ wide up to *300–400 (440) μ long* × *160–180 μ wide, rounded or blunt at apex*, the margin otherwise entire; lobule from 180–205 μ long up to *ca.* 200–320 μ long (including connate base) × 55–60 up to 60–80 (95) μ wide, *the free portion at most 45–60 (75–95) μ long, obtuse or* rounded at apex; *lobule length 0.65–0.85 of the length of lobe*; bracteole oblong, (225) 300–400 μ long × (110–150) 160–190 (225) μ wide, sharply incised for *ca.* ¼, the short lobes subacute *or obtuse to rounded*, 4–6 cells broad, the margins otherwise entire. Perianth at maturity *exserted for 0.6–0.7 of its length, narrowly obovoid, not or scarcely broader distally*, not or imperceptibly flattened, 480–620 μ long × 280–360 μ wide; *sharply 5-keeled to the middle, or below*, the keels crenulate, *rounded to the apex, not produced into shoulders*, the *beak long* and terminal, not or very slightly recessed; beak distally of slender, finger-like cells 7–10 × (25) 30–40 μ long; internal cells below apical ring of cells produced inwardly as finger-like papillae, occluding the perianth-mouth. Spores greenish, *ca.* 16 × 23–36 μ.

TYPE: Sanford, Seminole Co., Florida (*Rapp 64*).

DISTRIBUTION.—A neotropical species with a restricted range into the sub-

tropical portions of the southeast, ranging (probably) as far north as southern Georgia and Louisiana. Also known from Puerto Rico (Santurce; Mayaguez), and reported from Barbados (Pearson, 1922).

The species is polymorphic in certain respects, and is so easily confused with *L. minutiloba* that literature reports (except those of Evans, 1917) are probably quite meaningless. Typical *L. cladogyna* is known from the following stations:

Florida: LEE CO. (Caloosa [hatchee] R., *Austin*); SEMINOLE CO. (Sanford, *Rapp. 6, 19, 64!, 64a, 64b, 69*); ALACHUA CO. (Gainesville, *Nelson 104*); LIBERTY CO. (Alum Bluff, Appalachicola R., *RMS 33471, 33532, plate*); HILLSBOROUGH CO. (Hillsborough R. State Park, *RMS 33921*); COLUMBIA CO. (O'Leno State Park on Santa Fé R., n. of High Springs, *RMS 37268a, p.p.*, with *Cephaloziella obliqua* and *Phaeoceros laevis*; *RMS 37375, p.p.*, with *Cololejeunea setiloba, Frullania inflata*; *RMS 37280*; *RMS 37279a, p.p.*, with *Taxilejeunea obtusangula*; *RMS 37283, 37286a, 37289, p.p.*, with *Cololejeunea setiloba*; *RMS 37290*); HERNANDO CO. (Base of *Quercus nigra*, partly on sand, swamp 2–3 mi. s.e. of Brooksville, *RMS 22729*; typical phase with uniformly cladogynous perianths and normal bracts); DADE CO. (Primeval Mahogany-Royal Palm Hammock in wet sawgrass prairie, just s.w. of tip of Long Key Pineland, w. edge of Dade Co., in Everglades Ntl. Park, *RMS 37368b, p.p.*, among *L. laetevirens, Cheilolejeunea rigidula, Plagiochila hypnoides, Radula australis*); POLK CO. (Phosphate refuse bank, Highlands Pool near Lakeland, *Sharp, Dec. 1948!*; plants wholly elobulate, but wholly cladogynous, the bracts with large lobules, the innovations sterile but sometimes elongating!). **Mississippi:** S.W. GEORGE CO. (Wyatt Hills, n.e. of Ramsey Springs [Univ. of Mississippi Forest Lands] *RMS 26841*).

Redfearn (1952) reports *L. cladogyna* from seven Florida counties; I have tried to verify the accuracy of the reports. The Tennessee herbarium contained material from three counties; that from Dade Co. represents a small phase of *Rectolejeunea phyllobola*, with almost uniformly vestigially lobulate leaves, but with the broad and flattened androecia of that species. The report from De Soto County is of an elobulate-leaved plant which bears some gynoecia on elongate, leafy stems; the bracts have the small lobules of *L. minutiloba*, to which the material clearly belongs. The third report, from Polk Co. appears to be correct, and is cited above. Only three other collections, referred to *L. cladogyna*, widely distributed in exchanges, have come to light. One, determined as *L. cladogyna* was collected in Mexico by MacGregor, and is here referred to *L. calcicola* var. *mexicana*. The other ones, collected and determined by R. O. Schornherst Breen (Natural Bridge, Leon Co., *No. 2391* and Glen Julia, Gadsden Co., *No. 2258*) represent a submerged phase of *L. floridana*, as is readily obvious from the broad and transverse underleaves, and the large pellucid cortical cells averaging up to 48–51 μ broad.

There are two other reports of "Examinations" in Frye and Clark (1947); until the material is re-examined these reports are perhaps best deleted.

ECOLOGY.—*L. cladogyna* is one of the more mesophytic of the species of *Lejeunea*. It was originally reported to occur on "trees, logs and sandy banks" (Evans, 1918), but apparently occurs most often at the bases of tree-trunks, and on exposed roots in deeply shaded gullies or ravines, or in dense hammock forests. It is also occasionally present on moist decaying logs (then with *Lejeunea glaucescens, Aphanolejeunea evansii, Cololejeunea setiloba, Cheilolejeunea*

rigidula, *Ceratolejeunea laete-fusca*, and the moss *Fissidens radicans*, as in *RMS 33921*). In the writer's experience, the species is largely limited to sites with a permanently low light intensity, and with a high humidity. Several of the collections seen came from sites near flowing streams or springs. At the northern edge of its range (n.w. Florida, coastal Mississippi) the species occurs on springy banks, at the exposed bases and roots of trees, in dense shade, in a mature Beech-Magnolia forest, associated with *Magnolia macrophylla* (and/or *M. ashei*), *M. pyramidata*, *Kalmia latifolia*, *Illicium floridanum*, and other indicators of a mesic climax forest. The Mississippi plants occurred at the base of a large Beech tree, associated with *Lejeunea flava* and *L. laetevirens*, *Radula caloosiensis* (*langloisii* phase), *R. obconica*, *R. australis*, *Cheilolejeunea opaca* and *rigidula*, *Mastigolejeunea auriculata*, *Ceratolejeunea laete-fusca* and *Plagiochila ludoviciana*.

The species also occurs in somewhat more open forest along river banks, at the bases of *Betula nigra*, the River Birch. Here it is associated with *Cololejeunea setiloba*, and, higher up on the trunk, gives way to a more xerophytic community formed by *Frullania inflata*, *Leucolejeunea unciloba*, and *Cololejeunea minutissima* (*RMS 37225*). In similar situations found on the bases of *Taxodium*, or on the exposed "knees" of this tree, and over coral limestone adjacent to river beds. In such sites with *Taxilejeunea obtusangula*, *Phaeoceros laevis* and rarely with *Cephaloziella obliqua*. The plant, when in such rather open sites, more yellow-green, more compact, with imbricate and somewhat suberect and concave leaves, habitually rather different from the much more slender, shinier, deeper green phases of deeply shaded sites.

DIFFERENTIATION.—*L. cladogyna* is the smallest and most delicate of our species of the subgenus *Lejeunea*. Fully mature, fertile plants may be only 450–550 μ wide on the main shoots, although unusually robust plants occasionally attain a width of 750–850 μ or even 900 μ.[38] The species is characterized by the great delicacy, the distant to contiguous or barely imbricate, broadly rounded leaves (Fig. XLVIII: 10) which are strongly glistening and flat to concave when dry; the often diagnostically obovate leaves, in particular of juvenile shoots or shoot-sectors (Fig. XLVII: 10); the extremely small underleaves (usually considerably less than 150 μ wide (Fig. XLVIII: 8, 10, 13; the width 1.0–1.7 times the width of the stem); and the highly polymorphic lobules. The plants are frequently overlooked, even by the experienced collector, because they give the impression of being a somewhat impoverished or juvenile phase of a more "normal" species of *Lejeunea*. However, *L. cladogyna* appears to be an excellently characterized species, sharply separated from our other species by the combination of (a) virtually uniformly cladogynous inflorescences; and (b) nearly uniformly sterile subfloral innovations, which are usually limited in length. Unfortunately not even these primary criteria are wholly stable!

In its facies, especially the glistening appearance, small size, and particularly

[38] The plants of *Schuster 33471* (Liberty Co., Fla.) represent the small extreme; mature fertile plants are often only 480–550 μ wide, with the upper extreme 750 μ. The type plants range from 650–850 μ, very rarely up to 960 μ wide on mature leading shoots. The mature sterile shoots are no larger in this species.

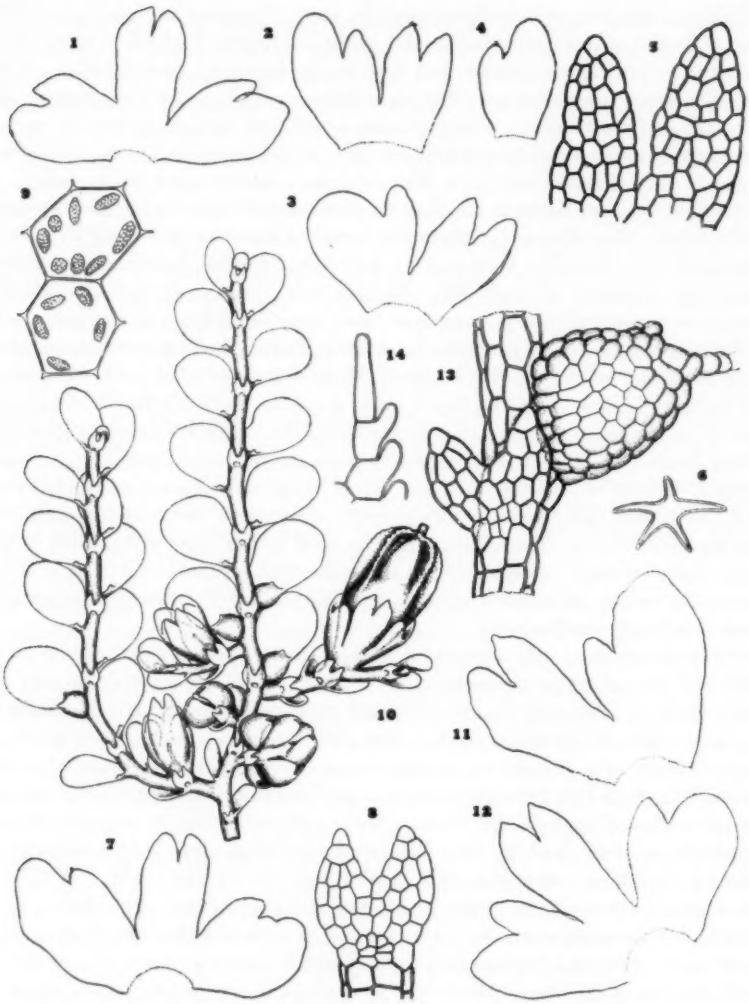
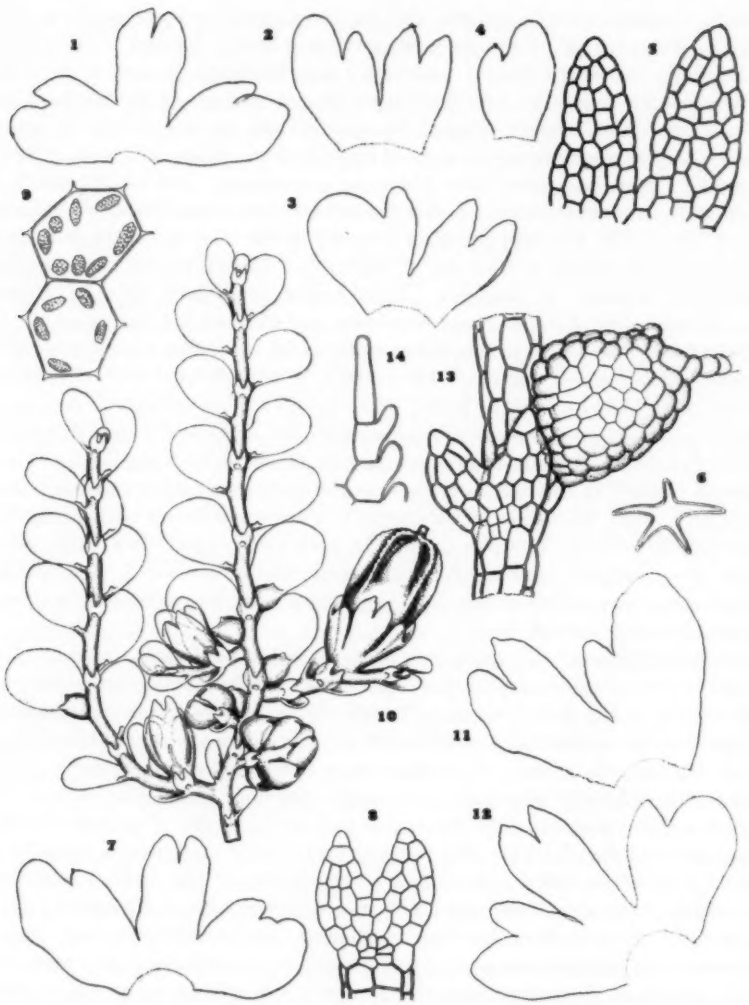


FIG. XLVIII. *Lejeunea cladogyna* Evs. 1, 4. Bracts and bracteole (×55); 2. Bract and bracteole (×65); 3. Bract and bracteole (×59); 5. Apex of unusually deeply bifid bracteole (×150); 6. Perianth cross-section (×57); 7. Ring of bracts and bracteole (×55); 8. Underleaf (×138); 9. Two median cells with oil-bodies (×640); 10. Fertile plant, wholly typical except for the single long innovation (×44); 11–12. Bracts and bracteoles (×95); 13. Underleaf and inflated lobule, *in situ* (×224); 14. Section through one side of beak of perianth (× *ca.* 350). (Figs. 1–8, from type, Sanford, Florida, *Rapp*; 9–14, from *Schuster 33471 and 33471a*, Liberty Co., Florida).

in the leaf-form (juvenile leaves often obovate; mature leaves with antical base not dilated, normally extending barely to stem midline and not extending across stem at all; leaf-apices always evenly and very broadly rounded) *L. cladogyna* very closely agrees with *L. minutiloba*. This similarity is confusingly great between *L. cladogyna* and the var. *heterogyna* of *L. minutiloba*, since both of these show dimorphic lobules, a few of which are always inflated and large (Fig. XLVIII: 10, 13). However, *L. cladogyna* has relatively few, and rather large and conspicuous, compound oil-bodies (Fig. XLVIII: 9), while those of *L. minutiloba* are numerous, homogeneous, glistening, and quite small. This difference, because of its absoluteness, is by far the most important one separating these two confusingly similar species. When dead material without oil-bodies is studied, the separation from *L. minutiloba* is a matter of great difficulty. *L. cladogyna* agrees with *L. minutiloba* in the general obsolescence of the lobules; in the tendency to develop obovate, subrotund leaves; and in general facies. However, all forms of *L. cladogyna* are separable from *L. minutiloba* in the form of the perichaetial bracts, and usually in the virtually constantly or constantly cladogynous gynoecia with small, weak sterile innovations. Further differential features appear to be (a) the presence, at least on occasional sterile branches, and particularly on sterile subfloral innovations, of leaves with well-developed, inflated lobules; unfortunately, forms of *L. cladogyna* occur with uniformly elobulate leaves, and forms of *L. minutiloba* occur with isolated lobulate leaves (thus greatly reducing the usefulness of this character, which, nevertheless, usually "holds"); (b) the smaller underleaves, little wider than the stem, whose lobes are rarely more than 4 (–5) cells broad; (c) female bracts with lobules usually almost as long as lobes, connate with them for most or almost all of their length (Fig. XLVIII: 1–4, 7, 12). This latter distinction, already emphasized by Evans, may unfortunately almost disappear with study of a series of cladogynous bracts. Evans (1917, pl. 24, fig. 12, 14) illustrates female bracts of *L. minutiloba* in which the lobules are fully two-thirds the length of the lobes; judging from their inferior size (to those figured in Fig. 6, 8, 9, 11) these bracts are from a cladogynous inflorescence. Such bracts can be matched in *L. minuta* var. *heterogyna*. The comparatively slender, obclavate perianths of *L. cladogyna* have the keels rather sharp and descending to below the middle of the perianth, or even the distal two-thirds of the perianth (Fig. XLVIII: 6, 10). In *L. minutiloba* and the var. *heterogyna* the keels of the much broader perianth are restricted to the distal one-third of the perianth.

A further distinction of some value lies in the perianth-shape. In *L. cladogyna* the keels are rounded distally, and the relatively elongate beak is strictly terminal. Exceptionally the distal portions of the keels are swiftly narrowed into the beak, with the consequence that the perianth is narrowly truncate at the apex, or even slightly emarginate (with the beak then slightly recessed). This is the case, for instance, in the plants from the Hillsborough R. (*Schuster 33921*). However, the plants of this collection retain the narrow perianth form characteristic of the species, with the width increasing only very slightly from the middle to the apex. They also show the high and long keels characteristic of the species.



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A further distinction of some value lies in the perianth-shape. In *L. cladogyna* the keels are rounded distally, and the relatively elongate beak is strictly terminal. Exceptionally the distal portions of the keels are swiftly narrowed into the beak, with the consequence that the perianth is narrowly truncate at the apex, or even slightly emarginate (with the beak then slightly recessed). This is the case, for instance, in the plants from the Hillsborough R. (*Schuster 33921*). However, the plants of this collection retain the narrow perianth form characteristic of the species, with the width increasing only very slightly from the middle to the apex. They also show the high and long keels characteristic of the species.

By contrast, all forms of *L. minutiloba* have the keels dilated distally, and suddenly contracted distally, resulting in the formation of rounded to angular shoulders, with the beak usually slightly recessed and much shorter. The distinct relationship of the two species is emphasized by the fact that in both species the cells below the terminal tier of the beak are produced, inwardly, as short finger-like papillae, more or less occluding the perianth-mouth (Fig. XLVIII: 14). However, the form of the beak and its terminal cells is very different in the two species. In *L. cladogyna* the beak is quite elongate and relatively slender; it has the cells of the terminal tier narrow, finger-like, with the rounded ends free. These cells are 30–40 μ long and only 7–8, occasionally 9–10 μ wide (i.e., usually about 4 times as long as wide). In *L. minutiloba* the shorter and stouter beak has variable terminal cells, which are always shorter and stouter. They are often only 15–21, occasionally up to 23–25 μ long $\times$ 8–10 μ wide at base, scarcely finger-like in form. *L. cladogyna* differs also from *L. minutiloba* in the nature of the subfloral innovations. These are, in *L. cladogyna*, usually short and do not develop more than 1–2 (rarely 3–6) pairs of juvenile leaves. In at least those cases where perianths are formed, the innovation remains permanently abbreviated (and its apex commonly decays, in the examined material). In those cases when an inflorescence is not fertilized, the innovation may become elongate and grow into a normal leafy shoot. Such an elongate, but sterile, innovation is shown in Fig. XLVIII: 10. In no case is such a cladogynous innovation again floriferous. By contrast, in *L. minutiloba*, the subfloral innovations are frequently again floriferous.

The segmented oil-bodies suggest several other regional Lejeuneae, among them *L. glaucescens* (and its relative, *L. calcicola*) and *L. longifissa*. The latter differs at once in the form of its bracts and underleaves. *L. glaucescens* and its relatives differ in the much smaller lobules of the female bracts; the almost free bracteole; larger underleaves, the larger leaf-cells; the much more robust size; the often elongate innovations which may be floriferous; the often longer and more conspicuous androecia. The virtually constantly cladogynous inflorescences are, perhaps, the most important feature separating *L. cladogyna* from these species (except for *L. glaucescens*, which normally also is cladogynous).

Lejeunea cladogyna can usually be separated from the three preceding species by cell size alone. The apices of mature leaves have cells usually averaging 15–17 μ, rarely 17.5–18 (19–20) μ on a few isolated leaves; the median cells quite consistently average below 23.5 μ wide (usually no more than 20–21 μ wide). In almost all cases the polygonal cells are extremely leptodermous, but in the material from the Hillsborough R. (*RMS 33921*) the cells are somewhat thick-walled, and vaguely defined trigones, and occasional intermediate thickenings are produced. In the cell-size, as well as leaf-shape, *L. cladogyna* again approaches *L. minutiloba*, rather than the preceding species to which attention was called.

RELATIONSHIPS.—Evans (1918) emphasized that he was unable to find any trace of acrogynous inflorescences in *L. cladogyna*, this feature giving the species its name. Prolonged study of several collections at first confirmed this finding, suggesting that *L. cladogyna* should be referred to the "genus" *Potamolejeunea*,

sensu Greig-Smith (1948), which is characterized by wholly cladogynous gynoecia, with the gynoecia either without innovations or with short sterile ones. *L. cladogyna*, in this feature, appeared to be wholly unique among our species of *Lejeunea*.

Prolonged search, however, eventually resulted in discovery (in *RMS 37268a*) of a single clearly acrogynous inflorescence! This was wholly typical and the bracts were no larger than is normal for the cladogynous inflorescences; their lobes ranged from 330–340 μ long × 170–180 μ broad, with the large lobules 280–310 μ long × 70–80 μ broad, free for the distal 0.25–0.35. The lobules were clearly united for up to one-third of their length with the bracteole, which was 365 μ long × 225 μ wide and incised for one-fourth its length by a closed sinus; the lobes were rounded. Even more significantly, acrogynous inflorescences were regularly demonstrable in the plants from Mississippi (*RMS 26841*). These plants are habitually identical with *L. cladogyna*, and I would assign them to this species without any reservations whatsoever. The plants possess free branching; small size (up to 750 μ wide); remote and dimorphic leaves, with inflated lobules in the minority; obovate lobes; shiny texture; great delicacy; small ventral cortical cells (20–28 μ wide); segmented oil-bodies; small underleaves (110–140 μ wide; lobes 3–4 cells broad); slender perianths with long carinae—all characteristics that, in their sum total, place the plants clearly as *L. cladogyna*. Furthermore, the cell size ranges well within the limits found in other collections: the marginal from 15.7–19 μ on many leaves, with some larger leaves with the marginal cells 20–21.5 μ; median cells 18–25 × 24–28 μ. The bracts and bracteoles are also wholly typical, although the bracteole is often nearly free; the bracts have in general lobules that are almost as long as the lobes (lobes 315–340 μ long × 180–185 μ broad; lobules 270–285 μ long × 85–95 μ broad; bracteole bilobed to one-fourth with blunt divisions, 295 μ long × 190 μ broad). Associated with these "typical" features we find that the highly ramified shoots show on many or most leading axes a single acrogynous inflorescence (the single innovation appears to be generally sterile, although rarely again fertile), but with the majority of inflorescences clearly on lateral branches. The cladogynous gynoecia are very variably situated. Most are, as is supposedly characteristic of the species, found on short, essentially leafless lateral branches; these may produce a short sterile innovation, but often produce an elongating innovation that may ramify; some of these branches of the innovation may produce androecia, and in isolated cases the branches of the innovation may produce secondary gynoecia! In other cases, the cladogynous gynoecial branches produce one to several pairs of normal vegetative leaves before the gynoecium is developed! In many cases the cladogynous fertile branches are pinnulate from an otherwise virtually unbranched leading shoot (as in Fig. XLVIII: 10); but often the leading stem produces sterile secondary stems of indefinite length, which in turn produce the fertile tertiary branches, both male and female. In some cases the secondary, leafy branches, although usually sterile if elongated, may produce a gynoecium; in a single case such a long, leafy secondary axis produced an androecium! These diverse positions of both gynoecia and androecia strongly suggest that the orienta-

tion of sexual branches, and the degree to which innovations are elaborated, to a large extent are associated with differences in vigor of the plant. Slender phases (which are most common, and on which Evans based the species) are uniformly cladogynous with short and sterile innovations. The less frequent, more robust phases show deviations in that the leading axis may be fertile, and the cladogynous lateral stems may produce highly elaborated innovations if fertilization does not take place! It may not be too fanciful to speak of differences, between populations, of the "élan vital," expressed in more robust plants by the greater vigor of the innovations, and retention, with development of branches, of enough vigor in the main shoot to produce gynoecia. Such an interpretation is borne out by the fact that *if* a cladogynous inflorescence remains sterile it often elongates (as in Fig. XLVIII: 10), but if fertilization takes place and a perianth is produced, the innovation remains abbreviated. In other words, the impression can hardly be avoided that a limited amount of energy is available; if this is used up in the formation of a sporophyte and perianths, not enough is left over to elaborate the innovations. Prolonged and painstaking examination of numerous individuals thus leads to the conclusion that the orientation of gynoecia and androecia is to a large extent determined by differences in vigor of the plants, induced probably in part by differences in the environment. This necessitates a conservative use of such criteria, and eloquently bespeaks the artificiality of trying to maintain the "genus" *Potamolejeunea* on any such basis as propounded by Greig-Smith (1948).

On the basis of the variability in position of the gynoecia, I concluded (in the preceding issue of this journal, pp. 130–132) that *Potamolejeunea* was not a natural genus as defined by Greig-Smith (1948). Certainly, "*Potamolejeunea*" *holtii*, which Greig-Smith transferred from *Lejeunea* to *Potamolejeunea* is not generically separable from *Lejeunea* s. str. However, as I have pointed out (*op. cit.*, p. 131), true species of *Potamolejeunea* possess a complex stem anatomy, involving numerous rows of cortical cells, with the ventral merophytes 4–6 cells wide in most cases. The stem anatomy of *Potamolejeunea orinocensis* and *P. temnantha* has been investigated subsequently and found to be of the "holostipous" type. Furthermore, these, typical species of *Potamolejeunea* possess leaf lobes that are united for almost their entire breadth to the stem; they possess delicate cells that tend to be elongated in and below the leaf middle. In spite of their delicacy, these species are very robust and up to 3–4 mm. broad. As a consequence, I would conclude, on the basis of studies subsequent to the publication of the preceding section, that there is good basis for maintaining *Potamolejeunea* as a discrete genus, on other bases than the gynoecial orientation. Furthermore, in *P. temnantha* the short, lateral gynoecial branches normally produce a short innovation that is almost immediately again fertile; the secondary gynoecium again may produce a fertile innovation. Sympodial "flower clusters" may then occur in this species. Thus, *if* we are to maintain *Potamolejeunea* as a genus, it must be based largely on the basis of stem anatomy and leaf-insertion, rather than on the absence of subfloral innovations. In any case, *Lejeunea holtii* is incorrectly assigned to *Potamolejeunea*. It is possible that this species, together with such

constantly cladogynous species as *L. corralensis* and *L. patagonica* should be segregated into a separate subgenus of *Lejeunea*. *Lejeunea inundata*, a South American species which Stephani transferred to *Potamolejeunea*, and which Evans (1935) retained in *Lejeunea*, has also been investigated in this connection. This species has a schizostipous stem anatomy, with seven rows of cortical and three rows of medullary cells. It differs at once in the subtransverse leaf-insertion and form of lobule, as well as in the very distinctive orientation of the gynoecia. Schuster and Kachroo (in sched.) will discuss this species, which forms a discrete new genus. At this juncture it is pertinent to note that (a) the circumscription of *Potamolejeunea* by Greig-Smith and Stephani is not tenable; and (b) although *Lejeunea cladogyna* almost always shows the cladogynous gynoecia of *Potamolejeunea* s. str., it bears no immediate affinity to *Potamolejeunea*. It is hardly possible that any distinct relationship between *L. cladogyna* and *L. holtii* exists, in spite of the similar orientation of gynoecia. The latter species differs from *L. cladogyna* in the ovate and somewhat bluntly pointed leaf-lobes; the homogeneous oil-bodies; and in the general lack of subfloral innovations.

Lejeunea floridana Evs.

Lejeunea floridana Evans, Bull. Torrey. Bot. Club **32**: 185, pl. 5, fig. 15–21, 1905; Schuster and Hattori, Jour. Hattori Bot. Lab. No. 11: 49, pl. X, fig. 9, 1954.

Plants in decumbent *pale or whitish-green* patches, or scattered and creeping among other Hepaticae. Plants relatively robust, *very pale, translucent, more or less glistening when dry*. on mature sterile shoots *mostly 1200–1400 (1525)* μ wide; stems prostrate, *120–150 μ in diam.*, rather sparsely and irregularly branched; ventral cortical cells *extremely large, averaging 40–50 μ wide* (on less robust, fertile stems, sometimes averaging only 36–40 μ; on extremely robust sterile shoots often 51–56 μ wide) × 52–60, occasionally *up to 90 μ long*. Leaves contiguous to weakly imbricate, *ovate* (near female bracts occasionally becoming dimidiate-ovate), on mature shoots from 500–540 μ wide × 650–680 μ long, *up to 630–680 μ wide* × *725–850 μ long*; antical margin straight or slightly curved near base, then strongly outwardly curved to the rather *broadly rounded (rarely obtuse) apex*; postical margin much less strongly curved; *margins distinctly crenulate* due to protuberant marginal cells. Lobules variable; often obsolete and reduced to a small basal fold, particularly on fertile plants; when well-developed, inflated and broadly triangular-ovoid, *inserted on stem by a very long line of insertion*, with a straight or slightly convex keel, 120–140 μ long × 90–125 μ wide, with the free margin involute to beyond apex, the apical sinus long and shallow, very oblique; juncture of keel and dorsal lobe usually obscurely and very obtusely notched (when lobule is inflated); apex of lobule formed by a single blunt cell, at whose proximal base is a hyaline papilla. Cells of lobe very leptodermous, slightly to moderately convex, quite pellucid, the marginal cells usually protuberant, *large:* marginal cells averaging *20–24 (27)* μ, near middle of leaf (23) *25–28 (34)* μ *wide* × *28–34 (36)* μ *long*; oil-bodies *segmented-papillose, large*: from 3.5 × 6–7 μ to 4–5 × 13–14 μ, rarely up to 3–4 × 16–17 μ, 4–10 (usually 5–6) per cell, fusiform to ellipsoidal, of fine, little protuberant spherules (0.5 μ or less). Chloroplasts small, usually 3.0–3.5 μ. *Underleaves relatively large:* varying from 200 μ wide × 150 μ long to 250 μ wide × 165–180 μ long, on very robust shoots 265 μ wide × 200 μ long, up to 350 μ wide × 255 μ long, *consistently averaging 1.3–1.4 (rarely to 1.5) times as wide as long*, transversely subrotundate, *distant, rounded to the base* but not cordate, the lateral margins evenly rounded, somewhat crenulate due to bulging cells; *lobes triangular, subobtuse to narrowly rounded at apex* (never tipped by 2 superimposed cells), the *sinus nearly closed*, sharp and acute; descending through 0.5–0.6 of the underleaf length; underleaf-cells *ca.* 16–22 × 21–25 μ; *rhizoid-*

initial disk often very distinct, formed of up to 24–34 cells (but occasionally of only 12–15 cells), the cells small (9–13 μ).

Autoecious. Androecia always *forming short lateral branches* of determinate length, *not proliferating apically*; *bracts small*, in 2–10 pairs, inflated, closely imbricate, forming usually a *narrow but compact spike*, subequally bilobed with rounded or obtuse lobes, the ventral lobes slightly smaller; 1–2-androus. *Female inflorescences variable in position*: sometimes on short lateral branches (which bear a single innovation), sometimes on leading leafy branches (which bear 1, rarely 2 subfloral innovations). Bracts obliquely spreading, *large*, *usually equalling or exceeding the mature perianth*; on inflorescences from leading branches lobes 680–720 (900) μ long × (35) 380–500 μ wide, obovate to oblong-lingulate, *broadly rounded distally*, entire-margined, except for the marginal crenulations; *lobule vestigial*, a minute fold 150–250 μ long × 40–65 μ wide, blunt at the apex, *united for virtually its entire length with lobe*. Bracts from cladogynous inflorescences often somewhat smaller, but still longer than leaves, their lobes 650–680 μ long × 330–370 μ wide; lobule only *ca.* 150 × 45 μ. Bracteole obovate, united with bracts for only a very short distance, 430–460 (600) μ long × 320–400 (430) μ wide, *sharply or obtusely incised for 0.1–0.25 of its length*, the very *short lobes broadly rounded* (rarely obtuse), the margins entire, except for the discrete crenulations. Perianth *not or barely exserted* beyond bracts at maturity, obovoid (but, exclusive of keels, widest near middle), 380–430 μ in diam. × 700–800 μ long, terete and cuneately narrowed towards base, the *upper fourth* sharply 5-carinate, the *keels dilated*, *usually extended upward as low*, *rounded or obtuse processes*, their margins distinctly crenulate due to protuberant cells; *beak slightly to distinctly recessed* (in extreme cases its apex considerably surpassed by the horn-like extensions of the keels); beak elongated, prominent (50–75 μ long), typically formed of 3 tiers of cells, *the distal finger-like*, *35–45* × *14–15* μ.

TYPE: Hammock, between Cutler and Camp Longview, in the Everglade Keys region, s. w. of Miami, Dade Co., Florida (*Small and Carter 1355*!).

DISTRIBUTION.—Restricted to Florida and to the West Indies, where it will probably prove widespread, although at present only known from Puerto Rico (Pagan, 1939).

Florida: DADE CO. ("Between Cutler and Camp Longview," *Small and Carter 1355!*, *type*, *1365!*); HILLSBOROUGH CO. (Hillsborough R., in Hillsborough R. State Park, *RMS 22872*, *33905c*); POLK CO. (Highlands Gully, Lakeland, *McFarlin 101!*; Highlands Pool near Lakeland, *Sharp*, *Dec. 1948!*); SEMINOLE CO. (Sanford, *Rapp 11–2863!*); MARION CO. (Juniper Creek, 4–5 mi. below Juniper Springs, *RMS 33400*); CLAY CO. (Ravine of Goldhead Branch State Park, *RMS 37209*, *37211*, *37212a and 37213 p.p.*, with *Riccardia stricta* and *Pallavicinia lyellii*); LEON CO. (Natural Bridge, submerged, *R. O. Schornherst Breen 2391!*, as *L. cladogyna*); GADSDEN CO. (Glen Julia, *R. O. Schornherst Breen 2258!*, as *L. cladogyna*).

The material from Leon County, cited above, represents a sterile phase with vestigial lobules; it is easily referable to *L. floridana* on the basis of the broad underleaves, which are wholly typically developed, and the large pellucid cortical cells, averaging up to 48–52 μ broad. The species was reported from Polk and Highlands Cos. by Redfearn (1952), but the University of Tennessee herbarium has only two specimens attributed to the species, both determined by Redfearn; one is an indeterminable scrap that cannot be referred here, the other is correctly identified (material from Polk Co., *Sharp*).

ECOLOGY.—The most mesophytic of our species of *Lejeunea*. Occurring occasionally as an epiphyte, at the bases of trees at the edges of streams, where the humidity is continually very high (as at Hillsborough R.; there with *Porella pinnata*), but more often on decaying damp logs, sometimes lying in water, or on

rich organic humus, over banks, along streams (as on Juniper Creek). When on humus, on the ground, the species commonly grows over *Lophocolea martiana* (type; Juniper Creek material), and occasionally over *Riccardia multifida*, *Pallavicinia lyellii*, *Telaranea*, etc. (Juniper Creek). It has been found, in its greatest abundance above flood level of the Hillsborough R. (*RMS 33905c*) on damp sandy soil over coral limestone, and on coral limestone, forming whitish, hyaline patches, associated with several *Plagiochila* species (*P. aspleniformis*, *P. floridana*, *P. ludoviciana* var.) and with *Cheilolejeunea rigidula*. The plant has much the same ecological distribution as *Crossotolejeunea bermudiana*, but is not restricted to limestone; it is more mesophytic than the latter, being found (in my experience), chiefly in areas closely adjacent to flowing water, at sites above or barely below the level of periodic submersion. Plants occurring near water (*RMS 22872*), although thoroughly typical in other respects, may show a uniform reduction of the lobules to vestigial basal folds, as is characteristic of *L. minutiloba*. However, in *L. floridana* this is a response to unusually moist conditions, while in *L. minutiloba* such rudimentary lobules are apparently genetically fixed, since they occur in plants from relatively dry rock walls.

DIFFERENTIATION.—*L. floridana*, when well-developed, is easily determinable. The species is robust, agreeing with or even surpassing *L. glaucescens* in size, often with a maximal width of about 1550 μ.[39] Like *L. glaucescens*, the plant is relatively delicate, with relatively large cells; however, the plants are much more whitish and pellucid than those of *L. glaucescens*. The leaves when moist are usually characteristically convex.

Sterile plants are easily recognized on the following bases: (a) underleaves averaging over 1.25 times as wide as long, on mature stems, quite evidently rounded at the base (Fig. XLIX: 2–4, 12), with the lobes somewhat crenulate, and obtuse or even narrowly rounded at the apex; (b) bases of the larger, mature underleaves with a well-developed, if irregularly defined, "disk," consisting of small, potentially rhizoid-developing cells; this is seen best on the terminal underleaves of robust, sterile shoots, where the very small cells of the disk (9–13 μ) contrast strongly with the larger underleaf cells (16–22 × 21–25, rarely up to 30 μ);[40] (c) in turn, the underleaf cells are very much smaller than the cells of the two ventral cortical rows of the stem; these vary considerably in size, depending on the robustness of the stem, but on main stems average at least 40–45 μ in width (Fig. XLIX: 12). The size of the cortical cells of the ventral merophytes is, by itself, absolutely distinctive—none of our other species of the genus having cortical cells attaining a width in excess of 35–38 μ, with the average width usually 25–32 μ. In *L. floridana*, robust shoots, bearing few inflorescences (as in

[39] Frye and Clark (1947) assign the species a width of 600–900 μ; this is certainly much too low; only juvenile plants measure less than 1000 μ wide.

[40] Frye and Clark (*op. cit.*, p. 807) state that the "appearance of having many small cells in the region in which . . . rhizoids arise . . . seems to us to be due to the holes or optical holes where the rhizoids originate rather than to small cells. Old underleaves show it, but those without rhizoids do not." In this conjunction, all that is necessary is to call attention of the student to Fig. XLIX: 2, 3, 12, in which young underleaves lacking rhizoids are shown.

RMS 22872), may have the cortical cells as much as 51–56 μ wide × 52–60, up to 90 μ long. In the slightly less robust type material, these cells average 48–52 μ wide × 50–70 μ long. By contrast, the much less well-developed, but freely fertile plants from Sanford, Florida (*Rapp 11*) show cortical cells only 36–40 μ wide × 64–85, rarely up to 90 μ long. As is so characteristic of all of the species of *Lejeunea* subg. *Lejeunea*, free fertility usually is associated with a gradual decrease in robustness.

The plants from the Hillsborough R. (*RMS 22782*) bear both male and female inflorescences; both appear to occur uniformly on short lateral branches, although the type material has perianths on elongate branches as well as on short ones. Even though Evans stressed the frequency of obsolete lobules, he was able to demonstrate occasional inflated lobules. In the present collection, the lobules are uniformly rudimentary—probably a modification associated with the extremely moist habitat. For that reason, the present material (as well as much material of *L. cladogyna*) would key to *L. minutiloba* Evs. in the work of Frye and Clark (1947, p. 793). It would appear that in most of the species of this genus, the size of the lobule is so variable that its use as a diagnostic character is quite unwarranted.

Lejeunea floridana occupies an isolated position in the genus, a fact stressed by Evans in the original description. Evans stated that the species is perhaps most closely related to *L. quinqueumbonata* Spruce which has been referred to *Otigoniolejeunea*, and shares with that species the obscurely produced and crenulate keels of the perianth—which are, however, not produced as horns, merely as low tumidities, between which the beak of the perianth barely protrudes. The separation of sterile material of this species from our other *Lejeunea* species is sometimes accomplished most easily because of the presence, at the underleaf-bases, of a sharply distinct radicelliferous area, occasionally forming a distinct disk, from which numerous rhizoids may spring (Fig. XLIX: 2–3, 12); in our other species of *Lejeunea*, there are usually fewer rhizoid-initial cells, forming a small and poorly defined field, never a disk. The ventral two rows of cortical cells in *L. floridana* are also unusually large, averaging 42–50–54 μ wide; in our other *Lejeunea* species they are much narrower, averaging 25–35 wide. Finally the underleaves, which are usually obviously less than twice the width of the stem, are quite distinctly wider than long, usually up to about 300–310 μ wide, and only 250–270 μ long; they always have a narrow sinus and usually obtuse or narrowly rounded lobes, terminated either by a single cell, or by two cells placed side-to-side (never superimposed). The oil-bodies are sometimes larger than in our other species of the genus, and relatively few per cell (4–10, usually 5–6); when well-developed they are narrowly fusiform or narrowly elliptical, occasionally narrowly ovoid, and from a minimum of 3.5 × 6–7 μ to 4 × 7.5 μ, to a maximum of 4.5–5 × 13–14 μ, occasionally nearly linear and 3–3.6 × 16–17 μ. Though larger than in our other species of *Lejeunea*, the oil-bodies are formed similarly to those of *L. flava*—i.e., composed of very numerous scarcely protuberant spherules (less than 0.5 μ in diameter), rendering the surface of the oil-body finely papillose (Fig. XLIX: 1). The chloroplasts average much smaller than the

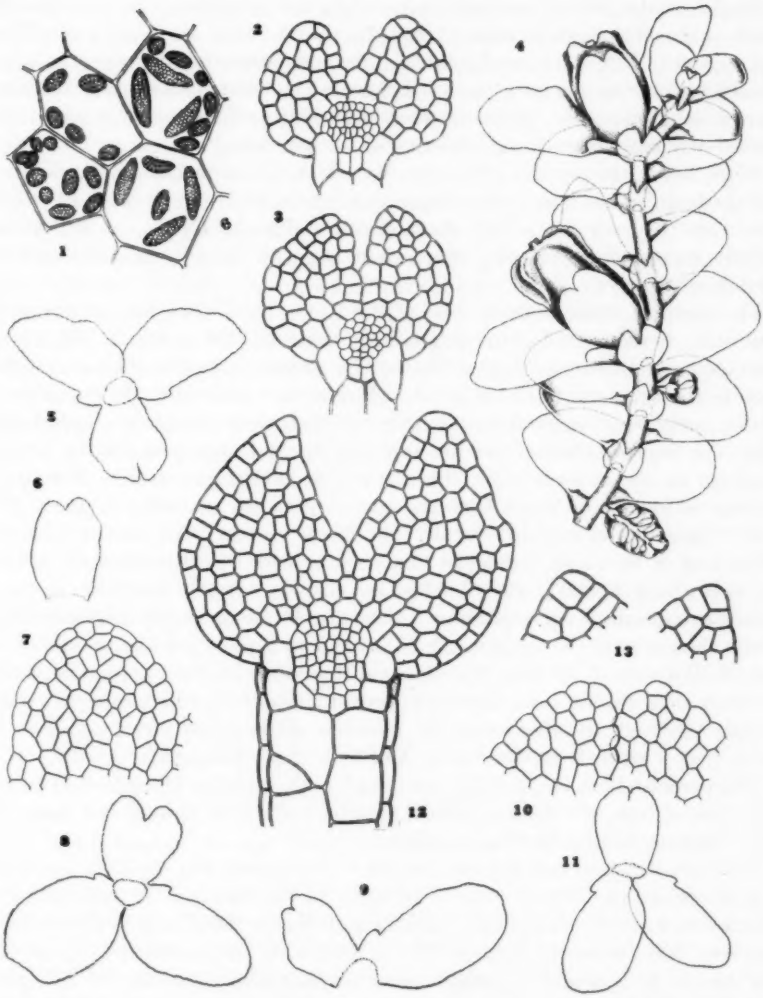

FIG. XLIX. *Lejeunea floridana* Evs. 1. Median cells (× *ca.* 500); 2–3. Medium-sized underleaves (×105); 4. Typical fertile shoot (×27); 5. Bracts and bracteoles (×27); 6. Bracteole (×27); 7. Lobe of bracteole in Fig. 8 (×120); 8–9, 11. Bracts and bracteoles (×27); 10. Apex of bracteole in Fig. 11 (×120); 12. Large underleaf, *in situ* (×142); 13. Apices of an underleaf (×142). (Figs. 1, 12–13, from *Schuster 22872*, Hillsborough Co., Florida; 2–3, 7–8, 10–11, from *Schuster 35400*, Marion Co., Florida; 4–6. 9, from *Rapp 11*, Sanford, Florida).

oil-bodies, in the present materials only 3–3.2 μ (i.e., averaging even less than the width of the oil-bodies). In some of the cells, the oil-bodies are broadly ellipsoidal and 4 × 6 to 5 × 7.5 μ, and some may even be spherical and about 4–5 μ in diameter; however, many or most cells always show the characteristic fusiform, narrow, long oil-bodies. Whatever the shape of the oil-bodies, they are always formed of equally minute and uniformly-sized spherules.

When fertile, the peculiar perianths, with the keels somewhat produced above, and the large bracts, longer than vegetative leaves, with minute or small lobules which are free only for a very short distance (Fig. XLIX: 4), as well as the slightly emarginate bracteole, whose short lobes are broadly rounded, are absolutely distinctive.

The specimen from Sanford, Fla. (*Rapp 11 = 2863*) does not appear to be typical *L. floridana*. It has the larger underleaves *ca.* 220 μ wide × 200 μ long; their median cells are *ca.* 22–25 μ. The ventral cortical stem cells are much smaller than in the type and in the Hillsborough material—only (33) 36–40 μ wide × 64–85, rarely 90 μ long, and considerably less than twice as wide as the underleaf cells. The female inflorescences are typically on an elongate or leading branch, innovate on one or both sides, the innovations often again fertile. The bracts average as long as or longer than the mature perianth (*ca.* 900 μ long × 380 μ wide, lingulate and rounded distally; the lobule reduced to a minute fold only 250 μ long × *ca.* 45–65 μ wide, free only at the very tip); the bracteole is large (*ca.* 600 μ long × 320 μ wide) bilobed less than ⅕ (usually less than 1/6), with obtuse to subacute, very broad lobes. The leaf-cells are unusually large on mature fertile shoots: near the margins 22–27 μ (20 μ in type), near the middle of the leaf 25–33 μ wide × 30–35 μ. The plants of this collection show typical perianths, however. The dilated keels sometimes spread laterally, in which case the beak is strictly terminal. In other cases, the horn-like dilations are erect, and then the short beak is strongly recessed (Fig. XLIX: 4, upper perianth).

The perianth beak is unusually prominent in this species, being formed usually of 3 tiers of cells, the distal of which is formed of a ring of long and finger like cells, often exceeding 40–42 μ in length.

Lejeunea floridana will key out neither in the generic key (p. 790), nor in the key to species (p. 793) in Frye and Clark. In the former it is separated from *Tarilejeunea* and *Rectolejeunea* as having underleaves 200 μ long × 230 μ wide or less (vs. "200 μ or more long and 300 μ or more wide" for the former two genera). On that basis, it would be sought under *Rectolejeunea brittoniae*. In the species key, the obsolete lobules of much of the material seen would result in its being sought under *L. minutiloba*, to which it is not closely related. The underleaves in the collection from Hillsborough River are larger than in the type material (in the latter described as 0.2 mm. long, with width not given). The plants differ from the type material further in the uniformly obsolete lobules—probably environmentally induced.

The writer believes that *L. floridana* is very immediately allied to the more tropical, extraterritorial *L. elliotii* Spruce. A specimen of this (Puerto Rico, *leg.* Heller) studied shows close agreement with *L. floridana* in the following points:

strongly crenulate leaf, underleaf and perianth-keel-margins; somewhat produced keels of the perianth; large, strongly elongate female bracts and bracteoles (the perianth not or scarcely emergent); bracts strongly crenulate, with rudimentary lobules; bracteoles very shallowly bilobed, strongly crenulate; ventral cortical cells of the stem large, up to 36–40 μ wide $\times$ 80 μ long or more; large and transverse underleaves. However, the bracts of *L. elliotii*, in some cases at least, are pointed and paucidentate, and the shallowly bilobed bracteole has sharp lobes and occasionally obscurely dentate margins; the perianth-keels are also crenulate with much more sharply produced, almost conoidal cells.

These differences almost disappear when a long series of the Hillsborough Co. plants (*Schuster 33905c*) is studied. In these the crenulations of the perianth-keels are as coarse and pronounced as in *L. elliotii*; the female bracts have occasionally lanceolate lobes that are slightly falcate and pointed, if obtusely so; the bracteoles are often bilobed to one-fourth (rarely deeper!) with a closed sinus and sometimes slightly overlapping, subacute to obtuse to rounded lobes. These plants suggest that, *if* the Puerto Rican plants of *L. elliotii* are typical of this species, *L. floridana* may well prove to be merely an extreme form of *L. elliotii*. Further study is clearly indicated.

APPENDIX

Subsequently to the completion of the treatment of *Lejeunea* subgenus *Lejeunea* a twelfth species of the genus has been collected, in northeastern Georgia. A treatment of this species shall appear in the next section of this revision.

PARMELIA CAROLINIANA NYL. AND ITS DISTRIBUTION[1]

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Species of the *Parmelia dubia* group, including several of the commonest foliose lichens, have minute cortical perforations (pseudocyphellae) as a constant anatomical attribute. A majority of the 1,440 specimens of that group examined recently (Culberson and Culberson, 1956) belong to *P. rudecta* Ach., a well-delimited species contributing only slightly to the total morphological and chemical variation of the *dubia* complex. Yet some specimens from the southeastern United States are superficially similar to *P. rudecta* but lack pseudocyphellae. Moreover, they do not contain lecanoric acid, a constant component of the latter species. Examinations in the Tuckerman Herbarium (Farlow Herbarium, Harvard University) revealed several identical specimens collected by H. W. Ravenel at Santee Canal, South Carolina. These specimens are authentic (isotypical?) material of *P. caroliniana* named by Nylander (1885).

[1] This work was supported by the Duke University Council on Research.

After its original description, *P. caroliniana*, reported from only a few additional stations, joined the ranks of so many American lichen species described by Europeans and then essentially forgotten. This obscurity must have resulted both from the similarity of *P. caroliniana* to the more widespread *P. rudecta* and from the infrequent lichenological collecting in the southeastern states. The records established by the misidentified herbarium materials found in the *dubia* group study, by specimens supplied by Dr. M. E. Hale, and by the author's collections from North Carolina now make it possible to delimit the distribution of *P. caroliniana*. Furthermore, a study of the chemical components ("lichen acids") of the species affords a reliable test, in addition to the morphological characters, for taxonomic determinations.

In both *P. caroliniana* and *P. rudecta* the upper cortex becomes yellow in the presence of an aqueous solution of potassium hydroxide. In *P. rudecta* the medulla is intensely red in contact with an aqueous solution of calcium hypochlorite but in *P. caroliniana* the reagent elicits no reaction. The use of Asahina's microchemical techniques demonstrated (Culberson and Culberson, 1956) that in *P. rudecta* the depside atranorin ($C_{19}H_{18}O_8$) causes the positive KOH test and that another depside, lecanoric acid ($C_{16}H_{14}O_7$), the positive $Ca(OCl)_2$ test. Microchemical tests to determine the substances in *P. caroliniana*, however, introduced complications.

Although the straight, needle-shaped, often macroscopic crystals of the quinoline salt of the depside perlatolic acid ($C_{25}H_{32}O_7$) were found in many preparations involving the glycerine-ethanol-quinoline mixture (Asahina, 1940), tests for atranorin in the glycerine-ethanol-*o*-toluidine solution, considered the most sensitive test for atranorin, gave only sporadic results. It seemed either that atranorin is present in low concentration in *P. caroliniana* or that another substance interfered in the crystallization of the *o*-toluidine salt of atranorin. The following procedure eliminated possible interference: Thallus fragments were extracted three times in a vial of 0.5 cc. of cold ethanol (in which atranorin is only sparingly soluble), the solution being decanted each time and replaced with fresh ethanol. The use of cold rather than of hot ethanol avoided decomposing any atranorin in the fragments. The decanted ethanol solution was evaporated, drop by drop, on a slide on a warming tray. A sticky, waxy residue resulted in contrast to the white crystalline residue from *P. rudecta*. The residue, scraped together and treated with the glycerine-ethanol-quinoline solution and heat, usually produced crystals of the quinoline salt of perlatolic acid within several hours to ten days. The glycerine-ethanol-quinoline test for perlatolic acid, however, must be subject to subtle technical difficulties not yet understood, because extracts of some plants gave negative results when first tested and positive tests with second extractions treated in seemingly the same fashion. If material was sufficient, all plants for which first tests were negative were retested. Dr. Y. Asahina of Tokyo confirmed the determination of perlatolic acid in quinoline preparations from a specimen supplied to him. This is apparently the second known *Parmelia* species containing perlatolic acid, the first being a variety of *P. cetrarioides* Del. (Asahina, 1952).

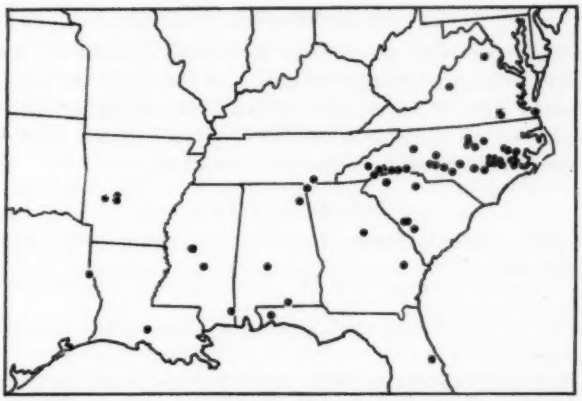

FIG. 1. The distribution of *Parmelia caroliniana* Nyl. in the United States.

Next, the thallus fragments were dried and re-extracted, directly upon slides on a warming tray, with acetone in which atranorin readily dissolves. The residue, scraped together and treated with the glycerine-ethanol-*o*-toluidine solution, produced crystals of the *o*-toluidine salt of atranorin within several minutes to several hours. Of the 75 collections studied of *P. caroliniana*, 64 (85 per cent) showed positive tests for both atranorin and perlatolic acid. Six (8 per cent) gave a positive test for atranorin and a negative one for perlatolic acid but were too small for repetition of the perlatolic acid test without depleting the herbarium sample. Five specimens (7 per cent), referred to *P. caroliniana* upon morphology alone, are so small that they were not tested at all.

Considering the apparent difficulties in tests for perlatolic acid, the results indicate that atranorin and most probably perlatolic acid are constant components of *P. caroliniana*. By differing not only morphologically but chemically from *P. rudecta*, *P. caroliniana* is well-established in its specific identity. A general survey of the *Parmeliae* of North Carolina will presently outline more precisely the relationships of the species to other members of the genus.

In Fig. 1, a map of the distribution of *P. caroliniana*, each dot refers to a station represented by one or more collections studied. The distribution of no other endemic southeastern lichen species has previously been mapped. The preponderance of records in North Carolina results from intensive collecting rather than from an exact coincidence of the state with the area of maximum frequency of the species although on the coastal plain *P. caroliniana* is one of the commonest lichens inhabiting the bark of *Pinus taeda* (Culberson, unpublished). Vainio (1926) cited the species from the State of Vera Cruz in eastern Mexico. In view of the total range of many vascular plants and bryophytes of the southeastern United States, Vainio's report is well within reason.

Thanks are expressed to Mrs. Chicita F. Culberson for her help in the preparation and in the examination of the microchemical tests.

SUMMARY

Parmelia caroliniana Nyl. differs from the closely related *P. rudecta* Ach. morphologically by lacking pseudocyphellae and chemically in producing perlatolic acid rather than lecanoric acid, a constant component of *P. rudecta*. *Parmelia caroliniana*, an endemic southeastern species, ranges from Virginia to Florida and westward through Arkansas and Louisiana.

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HYDROCOTYLE AMERICANA L. IN SOUTH CAROLINA

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Plants presumably representing the species *Hydrocotyle americana* L. have been reported from South Carolina previously. To quote Elliott (1821), *H. americana* "Grows in the mountains of Carolina". As Elliott was dealing with the plants of South Carolina and Georgia his mention of "Carolina" presumably means South Carolina. However, Elliott apparently had not collected *H. americana* in either of the Carolinas, since he said of this species, "My specimens are from Pennsylvania". Thus, reports of *H. americana* in the mountains of North Carolina, not South Carolina, may have been the sole basis for the inclusion of this species in Elliott's work, in which case the inclusion at that time was erroneous.

Ravenel (1883) lists *Hydrocotyle americana*, but did not annotate it "Mountains" or "In the mountains" as he usually did for montane species. Therefore it is quite possible that Ravenel's reference was to the *H. americana* of Walter (1788). *Hydrocotyle americana* Walter is a synonym of *H. ranunculoides* L., a lowland plant not uncommon in coastal areas of South Carolina.

More recently Coulter (1930), in a list of slightly over a thousand species of South Carolina plants "collected in three distinct regions of the state" includes *Hydrocotyle americana* L., and indicates that it occurs in the mountains (mountains, as here defined by Mrs. Coulter, means "in the Caesar's Head vicinity" of Greenville County).

Ives (1944), in a preliminary check list of the vascular plants of Greenville County, also includes *Hydrocotyle americana* L., but without comment as to locality other than "wet banks". Because of the close collaboration between Ives and Coulter there is a strong possibility that both authors are referring to the same locality or even to the same collection. However, despite the statement by Ives that "Preserved specimens of the species enumerated in this list are now on file in the herbarium of Furman University", such specimens are not referred to directly (e.g., by collection number) in his publication nor have they come to the attention of the various contemporary workers interested in *Hydrocotyle*, *per se*, or in the genus as an umbelliferous element of the South Carolina flora.

Thus it is not surprising to find that these early South Carolina reports of *Hydrocotyle americana*, the determinations of which cannot be verified by references to an actual specimen of the plant in question, have been disregarded. The manuals currently covering all, or portions, of the southeast, as do Mathias and Constance (1944–1945) in their treatment of the North American Umbelliferae, record the southern limit of this species as the mountains of North Carolina. Rodgers (1950), though citing the Coulter and the Ives publications (after searching in vain for the Ives specimen in the Furman Herbarium), also accepts North Carolina as the southern limit for *H. americana*. However, two collections of *H. americana* were made in the mountains of Pickens County, South Carolina, during the 1956 collecting season and this species may now be definitely counted as an element of the South Carolina flora.

The data for these collections are: Wet ditch at base of road bank, 3.1 mi. n. of U. S. Hwy. 178 on the Sassafras Mt. Rd., June 8, 1956, *C. R. Bell 3363*; Rocky Bottom Creek 2 mi. n. of Rocky Bottom, Aug. 22, 1956, *A. E. Radford 16534*.

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AN UNUSUALLY LARGE SPECIMEN OF *BERCHEMIA SCANDENS*

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A recent collection of *Berchemia scandens* (Hill) K. Koch was made in a low wooded area near a stream in Stanly County, North Carolina. The stem of this vine measured 9.45 in. in circumference, 2.4 in. in diameter, and was approximately 32 years old (determined by counting growth rings). No attempt was made to determine the over-all size of the plant at the time of the collection. The specimen, however, was well established with many other smaller specimens growing in the immediate vicinity.

A check of the literature revealed no data pertinent to the maximum size of the species. In fact, no records of any sort were found with which to compare this specimen. Those who have examined the specimen and who are familiar with the species have all stated that it is by far the largest they have ever encountered.

At present, an examination of the secondary xylem is under way to determine whether anything of special interest can be found to aid in explaining the unusual size.

The plant was collected in Stanly County, approximately 4.8 miles east-northeast of the city of Albemarle, N. C., near Little Mountain Creek. The collection (*Ahles 19982*) was made on September 24, 1956, by Harry E. Ahles with Robert S. Leisner on a trip made as a part of the Flora of the Carolinas Project now in progress at the University of North Carolina.

Voucher specimens and wood samples are on deposit at the Yale School of Forestry and at the University of North Carolina. Anyone interested in obtaining wood samples may do so by writing to the Botany Department of the University of North Carolina.

THE CHILDHOOD PATTERN OF GENIUS

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Genius by any definition is rare. If, following Galton, we make lasting fame one of the requirements, it is very rare indeed, and we are reduced to studying it at a distance through biography. Now, biographies have their limitations; as Havelock Ellis noted, one may search through them in vain for the most ordinary vital statistics. Above all, they cannot be expected to yield information on those details of early life, such as nursing and weaning and toilet training, to which

psychoanalysis has attached so much importance. When, therefore, one proposes as I do here to explore the question whether there is some pattern of environmental influences operating on children of genius which might help to account for their later achievement, it should be self-evident that the question is necessarily adjusted to something less than microscopic precision. Not only so, but, because the factor of heredity cannot be controlled, any answer whatsoever must be regarded as partial and tentative and ambiguous. Nevertheless, there may be some profit in asking the question, and insofar as it is directed simply toward the discovery of uniformity of environmental pattern there is no inherent reason why it should not be answerable, provided we do not insist on minute detail.

Table I presents the twenty geniuses into whose childhood this paper will inquire. The selection was partly deliberate, on theoretical grounds, and partly random, as will be explained. In Cox's monumental study of great geniuses (7) the main sample consists of 282 men drawn from the list of 1,000 which was compiled by J. McKeen Cattell on the principle that the amount of space allotted to them in biographical dictionaries could be taken as an objective measure of their true eminence. Though one may certainly quarrel with some of Cattell's results, the sifting process applied by Cox was admirable. She arrived at her smaller list by requiring: one, that the attained eminence should clearly depend upon notable personal achievement; and two, that the biographical material available should be sufficient to permit a reliable estimate of early mental ability. Men born before 1450 were eliminated. The chief task of Cox's investigation was to estimate the intelligence level displayed by these rigorously selected geniuses during childhood and youth. For this purpose the appropriate information was extracted from biographical sources and submitted to the judgment of three raters thoroughly experienced in the use of intelligence tests and the evaluation of IQ from behavior. Their three independent ratings, expressed as IQ's, were combined. Separate estimates were made for two periods of life: from birth to age 17, and from age 17 to age 26. As might be expected, the reliabilities of the estimates increased in proportion to the amount of biographical information, and, in general, the IQ's based on the more adequate material were higher. Consequently, one in search of illumination on the early environment of genius would naturally turn most hopefully to the geniuses in Cox's list who had been assigned the highest childhood IQ's. This I did. From her list I chose as my preliminary sample the 27 men whose IQ's in childhood had been estimated at 160 or higher. The final sample of 20, as given in Table I, was reached by dropping out those individuals for whom the biographical material in the University of North Carolina Library appeared to be inadequate.[1] As will be observed, the order of listing in the table is from the highest childhood IQ downwards. The reputation of each man is indicated in the column headed "Fame" by his rank number in Cox's sample, as based on Cattell. With respect to fame the sample appears to be a fair cross-section of Cox's larger group; with respect to IQ, as explained, it is highly selected. One sees at a glance that here are individuals who did extraordinary work in

[1] The seven omitted were Schelling, Haller, Wolsey, Sarpi, Constant, Brougham, Bossuet. In order to retain Leibniz an interlibrary loan was arranged for Guhrauer's biography.

TABLE I

	Estimated IQ in Childhood	Fame (Rank in 282)	Birth Order	Age at Marriage
J. S. Mill (1806–1873)	190	103	1 in 9	45
Leibniz (1646–1717)	185	19	Only	–
Grotius (1583–1645)	185	72	1 in 5	25
Goethe (1749–1832)	185	4	1 in 6	39
Pascal (1623–1662)	180	35	2 in 3	—
Macaulay (1800–1859)	180	53	1 in 9	—
Bentham (1748–1832)	180	181	1 in 2	—
Coleridge (1772–1834)	175	157	10 in 10	23
Voltaire (1694–1778)	170	2	5 in 5	—
Leopardi (1798–1837)	170	280	1 in 5	—
Chatterton (1752–1770)	170	163	3 in 3	—
Niebuhr (1776–1831)	165	135	2 in 2	24
Mirabeau (1749–1791)	165	30	9(?) in 11	22
J. Q. Adams (1767–1848)	165	274	2 in 5	30
Wieland (1733–1813)	160	152	1 in ?	32
Tasso (1544–1595)	160	48	3 in 3	—
Pope (1688–1744)	160	50	Only	—
Pitt (1759–1806)	160	9	2 in 5	—
Musset (1810–1857)	160	261	2 in 2	—
Melanchthon (1497–1560)	160	77	1 in 5	23

science, law, literature, or politics, and who fully deserve to be called geniuses. Their biographies should be relevant to the proposed question.

It should be understood from the outset that Cox did not neglect the problem of environment. Her biographical sketches furnish some very pertinent information, and she states as an important conclusion that, on the whole, youths who achieve eminence have superior advantages in their early days. Though she notes exceptions, she says: "The average opportunity of our young geniuses for superior education and for elevating and inspiring social contacts was unusually high. . . . The extraordinary training for leadership received by Pitt the younger, John Quincy Adams, Niebuhr, and the Humboldt brothers; the specialized instruction of Mozart, Weber, and Michelangelo undoubtedly contributed to the rapid progress of these great men among the great" (7, p. 216). The object of the present study is to push forward in the same direction of inquiry, but with more pointed attention to the social relations and their repercussions.

In Table I, one column briefly summarizes facts concerning order of birth. Considerable theoretical importance is sometimes attached to the chronological position of a child in the family. In particular, Galton, who was not prone to overemphasize environment, thought enough of order of birth to pay some heed to it in his investigation of British scientists; and he comments that "the elder sons have, on the whole, decided advantages of nurture over the younger sons. They are more likely to become possessed of independent means, and therefore able to follow the pursuits that have most attraction to their tastes; they are treated more as companions by their parents, and have earlier responsibility,

both of which would develop independence of character; probably, also, the first-born child of families not well-to-do in the world would generally have more attention in his infancy, more breathing-space, and better nourishment, than his younger brothers and sisters in their several turns" (13, p. 26). There is an intuitive appeal in the argument, but Galton does not support it by any precise analysis of his data. What may be said about the present sample? First, it must be admitted that there are several ways of stating the facts, depending on whether one includes or excludes half-siblings and siblings who died at an early age. The figures given in the table stand for full siblings and include all births. The half-siblings excluded in the three cases involved (Leibniz, Coleridge, Pope) were children by previous wives of their fathers. The impression produced by inspection is that there may be an excess of only and first children among these twenty geniuses. But an analysis of the probabilities does not favor this view very strongly. The average likelihood of being born in first place in the twenty families works out to about $\frac{1}{3}$, and the observed frequencies deviate from the theoretically expected only enough to yield a chi square of 2 in support of the hypothesis; since this corresponds to a confidence level of between .2 and .1 for the one degree of freedom, one is left in doubt. Pascal, Niebuhr, and Adams were first sons. If we estimate in terms of first sons, a total of 13, and adjust the probabilities to the expectation that about half the children in multiple births would be girls, the chi square is 1.8, again too small to support the hypothesis firmly.

Though the figures do not support a birth order hypothesis, there may nevertheless be something about position in the family which is significant. Let us look at the seven who do not rank as first-born children or first-born sons. Coleridge was born in his father's old age and was his "Benjamin"; Voltaire was so sickly during the first year of his life that there was daily concern over his survival, and his mother, an invalid, was incapable of having any more children; Chatterton was a posthumous child, and the previous boy in the family had died in infancy; Mirabeau was the first son to survive after the death of the first and a succession of girls; Tasso was the only surviving son, his older brother having died before he was born; Pitt was in the interesting position of being able to follow his father in a parliamentary career in the House of Commons, as his older brother could not do because of the inherited title; and Musset, the second of two sons, was younger than the first by a significant span of six years. When we weigh these additional facts, the general notion of some sort of positional effect begins to reassert itself.

One way in which position in the family might favor the development of a child would be by giving it higher attentional value for the parents. Close examination of the biographical data leads to the conclusion that these twenty men of genius, whether because of their position in the family or not, did as children receive a high degree of attention from their parents, as well as from others. In several cases it is clear that the attention exceeded that accorded to their brothers and sisters. Both very decided and very positive parental interest was displayed toward Mill, Leibniz, Grotius, Goethe, Pascal, Macaulay, Bentham, Coleridge, Niebuhr, Adams, Wieland, Pope, Pitt, and Melanchthon. Voltaire and Musset were far from neglected, but the attention bestowed upon them may have lacked

some of the intensity of focus notable in the preceding cases. If any of the children suffered comparative neglect or abuse, they would be Leopardi, Chatterton, and Mirabeau. Chatterton had no father from the time of his birth, and the fathers of Leopardi and Mirabeau were lacking in sympathy or worse. On the other hand, Chatterton's mother and sister helped him to learn to read, saw that he went to school, and were good enough to him that the promise he made them when a child to reward them with all kinds of finery when he grew up was fulfilled in the last year of his short life; Leopardi was provided with tutors and had access to his father's rich library; and Mirabeau, cuffed and persecuted as he finally was by his erratic father, was received into the world with an outburst of joy and was always provided for educationally, even though the arrangement may have been savagely disciplinary.

Favorable parental attention may take the two forms of displays of affection and intellectual stimulation. There is strong evidence for both in most of the cases in our list. Remarkable indeed are the educational programs followed by Mill, Goethe, Pascal, Bentham, Niebuhr, Adams, Wieland, Tasso, and Pitt, under the encouragement, guidance, and powerful insistence of their fathers. Yet it is not the educational program itself which requires our notice so much as it is the intimate and constant association with adults which it entails. Not only were these boys often in the company of adults, as genuine companions; they were to a significant extent cut off from the society of other children. The same statement can be made, on the whole, for others in the list whose educations proceeded less directly, or less strenuously under the guidance of fathers.

Warm attachments to children outside the family circle seem to have been rare, and there are several cases of isolation within the family, too. Yet it is within the family that most of the recorded intimacies between these geniuses and other children developed. Goethe, Pascal, Niebuhr, Macaulay, Voltaire, and Mirabeau experienced some intensity of affection for sisters; Musset for his older brother. Macaulay and Voltaire remained attached to their favorite sisters throughout their lives, becoming devoted uncles to their sisters' children; Goethe's and Pascal's affection for their younger sisters approached passion; and Mirabeau speaks of incestuous relations with his.

The reality and nature of the pattern to which I am pointing—the very great dominance of adults in the lives of these children, and their isolation from contemporaries outside the family and, sometimes, within—can be adequately appreciated only through a more detailed statement about each individual.

Mill, under his father's personal and unremitting tutelage, began hard intellectual work before he was three. From very early he was given the responsibility of acting as tutor to his brothers and sisters. This did not increase his affection for them. In fact, he came to share some of his father's own antipathy toward them and toward his mother. He explicitly states in his autobiography that his father kept him apart from other boys. "He was earnestly bent upon my escaping not only the ordinary corrupting influence which boys exercise over boys, but the contagion of vulgar modes of thought and feeling; and for this he was willing that I should pay the price of inferiority in the accomplishments

which schoolboys in all countries chiefly cultivate" (21, pp. 24f.) And again: "as I had no boy companions, and the animal need of physical activity was satisfied by walking, my amusements, which were mostly solitary, were in general of a quiet, if not a bookish turn, and gave little stimulus to any other kind even of mental activity than that which was already called forth by my studies" (p. 25).

Leibniz, his mother's only child, lost his father, a prominent university professor, when he was six. He retained two vivid memories of him, both of them expressive of the high esteem in which his father held him. His mother, who died when he was eighteen, devoted the remainder of her life to caring for him. He lived at home, free from "the doubtful liberties, the numerous temptations, the barbarous follies of student life" (18, p. 12). Before he was ten his father's carefully guarded library was opened to him, and he plunged into its treasures eagerly. It was conceivably no small thing to Leibniz that his father had regarded his christening as marked by a symbolic movement which seemed to promise that his son, as he wrote in his domestic chronicle, would continue in a spiritual and burning love for God all his life and do wonderful deeds in honor of the Highest (15, p. 4).

Grotius was close to his father. He signed his early poems Hugeianus, thus joining his own name Hugo with his father's name Janus or Joannes. At eight he reacted to the death of a brother by writing his father consolatory Latin verses. He had competent teachers at home, and entered the University of Leiden at eleven; there he dwelt with a devoutly religious man who impressed him deeply. He was famous in the literary world very early, and received high praise from distinguished men. He sought his father's advice when he chose a wife. One would infer from the limited evidence that his association from early childhood was primarily with adults.

Goethe throughout his childhood was carefully and energetically supervised in his varied studies by his father. He associated frequently with numerous skilled and learned and eminent men in Frankfort, among whom was his grandfather Textor. He enjoyed considerable freedom of movement through the city, in the intervals of his studies, and struck up several acquaintances outside the home among boys and girls; but these were certainly far outweighed by his adult contacts, and by his intimacy with his sister, who had much less freedom than he and who became increasingly embittered by the educational discipline of their father. In his autobiography he notes that he was not on friendly terms with a brother, three years younger, who died in childhood, and scarcely retained any memory of the three subsequent children who also died young. How close he and his sister were may be gauged by these words regarding the after-effects of his love-affair with Gretchen, at about fourteen: "my sister consoled me the more earnestly, because she secretly felt the satisfaction of having gotten rid of a rival; and I, too, could not but feel a quiet, half-delicious pleasure, when she did me the justice to assure me that I was the only one who truly loved, understood, and esteemed her" (14, p. 192).

Pascal was so precious in the eyes of his father, after his mother's death when

he was three, that, as the older sister tells us, the father could not bear the thought of leaving his education to others, and accordingly became and remained his only teacher. At eighteen Pascal's health broke down from ceaseless application. He was frequently in the company of the learned men surrounding his father. His primary emotional attachment was to his younger sister, Jacqueline; her religious retirement strongly influenced his own religious development.

Macaulay early became absorbed in books, but his studies were more unobtrusively guided by his father and mother and other relatives than in the cases preceding. He was especially attached to his mother in early childhood, and at home among his brothers and sisters was overflowingly happy and playful. A sister writes: "He hated strangers, and his notion of perfect happiness was to see us all working round him while he read aloud a novel, and then to walk all together on the Common" (30, p. 67). He was reluctant to leave home for school for even a single day, and he was acutely homesick when placed in a boarding school at about twelve; there, though tolerated and even admired by his fellow pupils, he had little to do with them, living almost exclusively among books. The children at home passionately loved him. It should not be overlooked that his father was a deeply religious man of great force of character, energetic in religious and political reform movements of considerable scope.

Bentham's father, ambitious to make a practical lawyer of his first and for nine years his only child, kept him to a rigorous schedule of instruction in everything from dancing and military drill to Greek from a very early age. From seven to twelve he spent the winters at a boarding school, which he did not enjoy; in the vacations at home his schooling, under private tutors, was much more intensive. He was happiest on visits to grandparents in the country, where he could talk to an old gardener or climb up in a tree and read a novel. Too small and weak to win the admiration of his fellows, "he tried to be industrious and honest and noble and dutiful, finding that such a course brought praise from his elders" (10, pp. 20f.). When the death of his warmhearted mother desolated his father and himself, Jeremy "was just turned twelve, and was ready for Oxford, if a frail and undersized boy of twelve could be said to be ready for anything" (10, p. 22).

Coleridge's father, though unambitious in general and not very attentive to the education of his numerous other children, took special pride in him and endeavored from the beginning to prepare him for the Church. Coleridge was the last of fourteen children (ten by his mother), and the extreme fondness of his parents aroused the hostility of the older boys toward him. They drove him from play and tormented him. On one occasion, when he was eight, he ran away from home after a ferocious combat with the brother whom he had displaced as baby of the family; he was found only after a prolonged search, and he remembered all his life the tears of joy on his father's face and his mother's ecstasy when he was recovered. Death of the father, when he was nine, deprived him of his most valued companion. Shortly afterwards he was sent to a charity school in London. Here he made a few friends, notably Lamb, but he lived a great deal in books and in his own imagination.

Voltaire was born five years after the death in infancy of the next preceding

child, and his own life was despaired of daily for the first year. His mother was an invalid; his father was a busy lawyer and does not seem to have concentrated any particular attention on him, beyond desiring that the boy should himself be prepared for the law. His education at home proceeded under the guidance of three distinguished and learned men, particularly the Abbé Chateauneuf, his godfather. The two other surviving children were considerably older than he; the brother he disliked, but he was fond of his seven-years-older sister, and, after his mother's death when he was seven, it was she to whom he was chiefly attached in the family. At ten he was quartered in the best Jesuit school in France by his ambitious and wealthy father; here he made the warmest and most lasting friendships in his life, but they were with the teachers rather than with the boys.

Leopardi, the oldest of five children, remained until he was twenty-four, practically immured, in the house of his father, the Count, in a town which he despised. In Leopardi's own words: "Had no teachers except for the first rudiments, which he learned under tutors kept expressly in the house of his father. But had the use of a rich library collected by his father, a great lover of literature. In this library passed the chief portion of his life, while and as much as permitted by his health, ruined by these studies; which he began independently of teachers, at ten years of age, and continued thenceforth without intermission, making them his sole occupation" (29, p. 2). His closest companion was his brother Carlo, a year younger; but he was reticent even with him. With the other children he liked to produce plays in which the tyrant (his father) was worsted by the hero (himself). At a later age he regarded his home as a prison from which he had to break out.

Chatterton, born three months after his talented father's death, was the second surviving child of his very young mother, who had borne her daughter four or five years earlier before her marriage was legalized. Under their instruction, he learned the alphabet from an old illuminated music manuscript of his father's, which his mother had been about to throw away, and learned how to read from an old blackletter Testament. He had been dismissed from his first school as a dullard. Later, he went to the uninspiring charity school which had been attended by his father. A note on his relations with playmates before he was five speaks of him as "presiding over his playmates as their master and they as his hired servants" (20, p. 22). Already at five he was greedy for fame, and asked that a cup which had been presented to him by a relative should have on it "an angel with a trumpet, '*to blow his name about*,' as he said" (20, p. 23). He did form friendships at school, one in particular; and the death of this boy plunged him into melancholy. But with none of these, or with his sister, was he intimate enough to share the secret of his Rowley poems, those impressive forgeries which seem to have been written under the inspiration and tutelage of the beautiful church of St. Mary Redcliffe rather than any human preceptor.

Niebuhr's father, who had been a military engineer and explorer, took up residence after his marriage at forty in a retired little town and devoted himself to his wife and family of two children. He liked to entertain his own and other children with stories, games, and music; but he concentrated particularly on the

instruction of his son, for whom he also provided tutors from about four or five. A cultured neighbor, Boje, who was editor of a literary periodical, took much interest in the boy; and Boje's wife began his instruction in French. Her death when he was ten overwhelmed him with grief and inclined him even more seriously to his studies. Between fourteen and eighteen he spent most of the day in hard work and general reading. When he was sixteen his father, thinking that his attachment to home was excessive and that he was studying too much alone, sent him off to a school in Hamburg in the hope that he would become more sociable; but he was unhappy, and insisted on coming back. From an early age ill health and his mother's anxiety contributed their share to his inclination to solitude.

Mirabeau, the first surviving son of a family of the nobility, was in the beginning his father's pride. Later, after disfigurement by smallpox at three and displacement from the position of only son by the birth of a brother when he was five, he became increasingly the object of his erratic father's dislike. Intense marital discord made him the more hateful because he resembled his mother's side of the house. He was unfavorably compared with the other children, and repeatedly put under severe disciplinarians as tutors. Eventually his father had him imprisoned more than once. In the face of this persecution, helped partly by the affectionate interest of an uncle, Mirabeau succeeded nevertheless in developing an extraordinarily winning manner in speech and personal contacts, even charming his jailers into relaxing their punishments. Whether or not he was inclined to solitude, it was forced on him by his father; much of his learning and literary production took place in prisons or their equivalents. He was highly erotic, and may have had sexual relations with his younger sister; for so he asserts.

Adams regarded even his name, John Quincy, which was his great-grandfather's, as a perpetual admonition to live nobly. The Revolutionary War and the battle of Bunker Hill, which he witnessed, confirmed a serious habit of mind from early childhood. As his father was absent from home a great deal, he was already as a small boy depended upon by his mother as if he were a man. His education commenced at home under a tutor, and continued in Europe in the company of his father and other men notable in the governmental service. It was not until he entered Harvard that he attended a regular school for any length of time. Both his mother and his father tried to keep him from the corrupting influence of other boys, and it is evident from the nature of his life that his chief contacts were with grown men of serious and intellectual character. He read a great deal under the guidance of his father, whom in his earliest letters he obviously wished to please.

Wieland was educated at home under the eyes of his father, a pastor, in somewhat the same severe manner as was Goethe. He studied hard from three years of age. He says of his childhood: "I was deeply in love with solitude and passed whole days and summer nights in the garden, observing and imitating the beauties of nature" (26, p. 19). He was much more attached to books than to people. Prior to age seventeen, says his biographer, "We encounter not a single friend of his own age, only books and those who helped with them!" (26, p. 24).

He was sensitive and unsociable when away at school, and when he returned home he lived alone or associated only with older men. His biographer makes no mention of his relations with his several siblings.

Tasso, whose old father was often compelled to be away from home, lived with his young mother and his sister until he was separated from them forever at ten, to join his father at the court of his patron prince. Even while he remained at home he was being strictly educated, first by an old priest, and then in a Jesuit school, which he loved. His mother, of whom he was passionately fond, died two years after he went to join his father. Of his childhood, Boulting says: "The prolonged absences of his father, the tears of his mother, the straitened circumstances and this sudden death were not healthy influences for a sensitive lad, and there was a great deal too much educational pressure put upon him. Bernardo was proud of Torquato's talents and ambitious as to his future. He forced him on and took scudi from a slender purse to pay for special lessons in Greek. But a cousin came to Rome from Bergamo to share in Torquato's studies. No bookworm was this lad, but full of fun and a thorough boy. Nothing could have been luckier" (3, p. 31). A little later he had as his companion in the study of the graces (horsemanship, jousting, etc.) a boy of eight, son of Duke Guidobaldo. Otherwise he seems to have associated primarily with men, often men of great dignity and learning.

Pope, the only child of his mother (there was a half-sister more than nine years older), was from the earliest period a domestic idol, as Stephen says. His father and mother, both forty-six at his birth, and a nurse, concentrated their affection upon him, which must have been all the more intense because he was sickly, and humpbacked like his father. "The religion of the family made their seclusion from the world the more rigid, and by consequence must have strengthened their mutual adhesiveness. Catholics were then harassed by a legislation which would have been condemned by any modern standard as intolerably tyrannical" (28, p. 2). Most of his education was accomplished at home, with some help from a family priest and his father, who corrected his early rhymes. From twelve he threw himself into his studies so passionately that his frail constitution threatened to break down.

Pitt was born at the high peak of his father's career as Prime Minister of England. When the title of Earl of Chatham was conferred on him, this second son, then seven, exclaimed, "I am glad that I am not the eldest son. I want to speak in the House of Commons like papa." Partly because of his feeble health, the boy was brought up at home under the instruction of his father and a tutor. His father concentrated upon developing his oratorical powers. At fourteen he was sent to Cambridge, where he was placed in the care of a sound scholar, who remained his inseparable companion, and practically his only one, for more than two years. He had no social life there. He read with facility such books as Newton's *Principia* and the obscurest of the Greek poets. "Through his whole boyhood, the House of Commons was never out of his thoughts, or out of the thoughts of his instructors" (17, p. 129).

Musset was the second son in a family devoted to literature, "an infant prodigy

on whom the intelligence of his brother, six years his elder, did not fail to exercise a stimulating effect. Alfred developed his mind in the constant companionship of Paul much more rapidly than he would have in the company of children his own age" (5, p. 12). He was notable from early childhood for his sensitivity, charm, emotional ardor, dramatic power, and susceptibility to feminine beauty. At a very tender age he was already disappointed in love. He went to school for a short time with his brother, but sickness and the hostility of the other children toward these Bonapartists soon led to their being tutored at home, by a young man who knew how to combine pleasure with instruction.

Melanchthon always remembered the dying injunction of his father: "I have seen many and great changes in the world, but greater ones are yet to follow, in which may God lead and guide you. Fear God, and do right" (25, p. 6). Before this time (his father died when he was eleven) he was, by his father's express wishes, strictly educated, for a while in a local school, and then by a tutor, a conscientious teacher and stern disciplinarian. Afterwards, he came more directly under the influence of the celebrated scholar Reuchlin, who was his relative. It was Reuchlin, impressed by the scholarship of the little boy, who changed his name from Schwartzerd to its Greek equivalent Melanchthon. Of his earlier childhood it is related that he often gathered his schoolfellows around him to discuss what they had been reading and learning; and his grandfather delighted to engage him in learned disputes with traveling scholars, whom he usually confounded.

The brief sketches preceding tend to confirm the rule, I believe, that children of genius are exposed to significantly great amounts of intellectual stimulation by adults and experience very restricted contacts with other children of their age. Nor should we overlook the fact that books themselves, to which these children are so much attached, are representatives of the adult world. This is true in the superficial sense that they are provided by adults and, more significantly, may be drawn from a father's sacred library (one thinks of Leibniz, Leopardi, even Chatterton); it is true in the profounder sense that they are written by adults, and, in the case of most of the reading done by these children, *for* adults. Books extend the boundaries of the adult empire.

There is an effect of this constant intercourse with the adult world which may be especially important in the development of genius. Not only is there an increase of knowledge, which is the usual aim of the instructors; there is also, in many cases, a profound excitement of imagination. Even John Stuart Mill confesses that he did not perfectly understand such grave works as the more difficult dialogues of Plato when he read them in Greek at seven. What, then, happens to such adult material pouring into the child's mind? Mill does not elucidate his own case; but there is evidence in a number of the biographies before me that the dynamic processes of phantasy go to work on it and richly transform both what is understood and what is not.

Much of Goethe's association with other children was simply an occasion for expressing his vivid phantasy life; he entranced them with stories of imaginary adventures. Musset, also, reveled in a world of make-believe based upon the

Arabian Nights and similar literature, and bewitched his enemies by the magic power of imagination. These were to become poets. But Bentham, who was no poet, imagined himself growing up as a hero like Fénelon's Telemachus and was stirred to moral fervor by sentimental novels. And two of the practical politicians in the list, Pitt and Niebuhr, may give us some insight into the process. When Pitt was around thirteen or fourteen he had written a tragedy, of which Macaulay has this to say: "This piece is still preserved at Chevening, and is in some respects highly curious. There is no love. The whole plot is political; and it is remarkable that the interest, such as it is, turns on a contest about a regency. On one side is a faithful servant of the Crown, on the other an ambitious and unprincipled conspirator. At length the King, who had been missing, reappears, resumes his power, and rewards the faithful defender of his rights. A reader who should judge only by the internal evidence, would have no hesitation in pronouncing that the play was written by some Pittite poetaster at the time of the rejoicings for the recovery of George the Third in 1789" (17, pp. 68f.). Out of his learning Pitt had constructed a dream prescient of his own future career. And who can say that the actions of a Prime Minister are not as much the expression of a private drama as they are the realistic application of the sciences and the laws? Niebuhr, who became a practical man of business and politics as well as the historian of Rome, writes explicitly about his own childhood experience, in a letter to Jacobi in 1811: "Our great seclusion from the world, in a quiet little provincial town, the prohibition, from our earliest years, to pass beyond the house and garden, accustomed me to gather the materials for the insatiable requirements of my childish fancy, not from life and nature, but from books, engravings, and conversation. Thus, my imagination laid no hold on the realities around me, but absorbed into her dominions all that I read—and I read without limit and without aim—while the actual world was impenetrable to my gaze; so that I became almost incapable of apprehending anything which had not already been apprehended by another—of forming a mental picture of anything which had not before been shaped into a distinct conception by another. It is true that, in this second-hand world, I was very learned, and could even, at a very early age, pronounce opinions like a grown-up person; but the truth in me and around me was veiled from my eyes—the genuine truth of objective reason. Even when I grew older, and studied antiquity with intense interest, the chief use I made of my knowledge, for a long time, was to give fresh variety and brilliancy to my world of dreams" (4, p.354).

My point is that phantasy is probably an important aspect of the development of genius, not only in those cases where the chief avenue to fame is through the production of works of imagination in the ordinary sense, but also in those where the adult accomplishment is of a different sort. Instead of becoming proficient in taking and giving the hard knocks of social relations with his contemporaries, the child of genius is thrown back on the resources of his imagination, and through it becomes aware of his own depth, self-conscious in the fullest sense, and essentially independent. There is danger, however, in the intense cultivation of phantasy. If it does not flow over into the ordinary social relations by some channel, if it has to

be dammed up as something socially useless, then it threatens life itself. An expression of what I am referring to is given in that powerful scene in the first part of Goethe's *Faust* where the physician-magician, tampering with incantations, raises a spirit of overwhelming presence and quails before him. Something nearer to an outright demonstration is furnished by the life of Chatterton and his suicide.

Before he was eighteen Chatterton was dead by his own hand. If we examine his life, we see that it breaks apart into two distinct regions: an outer shell of schoolboy, apprentice, pretended antiquarian, and writer of brittle satire; and a core—the serious and deeply emotional 15th century poet Rowley, whose connection with himself he never publicly acknowledged. One must not forget that Chatterton's phantasy existence as Rowley has points of contact with his father, the musician schoolteacher who died before his son was born, but who, in a sense, presided over the boy's education through the music manuscript from which he learned his letters and the blackletter Testament in which he learned to read, and who, by his connection and the connection of his family with the magnificent church of St. Mary Redcliffe, which overshadowed the place of Chatterton's birth and was his favorite resort from the brutalities of Bristol, might surely continue to hold converse with the imaginative boy. The Rowley poems furthermore are related to Chatterton's search for a pedigree. In short, through Rowley, Chatterton established relations with the world of the dead; and since he could not admit that he himself was the author of the Rowley poems, but had to pretend to have found them in his role as antiquary, and was thus rejected as an impostor by Walpole, he could not through Rowley establish contact with the world of the living. The surface which he was able to present to the world was hard, brittle, violent, unreal. Yet even in his relations with the world he appeared to be doing the same thing he was doing through the Rowley phantasies, namely, seeking a father to love and protect him. He evidently placed great hopes in Walpole; but he had also tried and been disappointed in the patronage of men of lower caliber in Bristol. Eventually he came to a dead end in London, where he had no friends even of the quality of Bristol's Catcott. Just before he committed suicide he was Rowley once again in the most beautiful of his poems, the *Balade of Charitie*, which sums up his experience of the world and his yearning for a loving father. If it was Rowley who enabled Chatterton to live, it was also Rowley who opened the door of death for him and ushered him out of a world of constant bitter disappointment into a world of kindly and Christian spirits.

Chatterton is a supreme example of the dangers and costs of genius. Having no father or other appreciative adult to link him to the world, he was swallowed up by his imagination. But it is too often overlooked in the textbooks that genius in less tragic cases is generally a costly gift. Superficially an enviable piece of luck, it is actually a fatality which exacts tribute from the possessor. Extreme absorption in very hard work is one of the penalties, and sometimes broken health. Isolation from contemporaries, often increasing with the years, is another. Whether we should include heterosexual difficulties as another, I am not sure, but I have indicated some of the facts in the last column of Table I and wish to consider the

matter briefly. Fifty-five percent of our sample did not marry at all. There may be no special significance in this, since according to statistics for the United States (11) the marriage rate for the total population of males above fifteen is only about 60 per cent and may have been lower in earlier times. On the other hand, this group, with the exception of Chatterton, ranges in age from 39 to 84 and should be compared with the higher age groups. According to the 1930 census in the United States marriage had been entered into by 86 per cent of men in the age range from 35 to 44, and by age 60, which is about the median for our group of geniuses, it had been entered into by about 90 per cent. I will only note further that some delay or reluctance or dissatisfaction attended the marriages of Mill, Goethe, Coleridge, Mirabeau, Wieland, and perhaps Melanchthon, but it would not be desirable here to go into greater detail because of the impossibility of making appropriate comparisons. It may be that for marriages both freely contracted and happily sustained a rate of 3 in 20 is not out of the ordinary, though I should be inclined to say that here too we have an expression of the costliness of genius.

In summary, the present survey of biographical information on a sample of twenty men of genius suggests that the typical developmental pattern includes as important aspects: (1) a high degree of attention focused upon the child by parents and other adults, expressed in intensive educational measures and, usually, abundant love; (2) isolation from other children, especially outside the family; and (3) a rich efflorescence of phantasy, as a reaction to the two preceding conditions. In stating these conclusions I by no means wish to imply that original endowment is an insignificant variable. On the contrary. Galton's strong arguments on behalf of heredity appear to me to be well-founded; and in this particular sample the early promise of these very distinguished men cannot be dissociated from the unusual intellectual qualities evident in their parents and transmitted, one would suppose, genetically as well as socially to their offspring. It is upon a groundwork of inherited ability that I see the pattern operating. Whether the environmental phase of it summarized under (1) and (2) is actually causally important, and to what extent the environmental factors are related to the blossoming out of phantasy, are questions which could be examined experimentally, though obviously any thorough experiment would require both a great deal of money and a certain degree of audacity. It might be remarked that the mass education of our public school system is, in its way, a vast experiment on the effect of reducing all three of the above factors to minimal values, and should, accordingly, tend to suppress the occurrence of genius.

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